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STUDIES ON RDX AND RELATED COMPOUNDS

IX. DETERMINATION OF NITRIC ACID AND ACETIC ANHYDRIDE IN BACHMANN LIQUORS[1]

BY R. H. BETTS, R. S. STUART, AND C. A. WINKLER

ABSTRACT

An electrometric method for determination of nitric acid in RDX(*B*) liquors has been developed. The method is not largely affected by ammonium nitrate and acetic anhydride. A precision of ±0.5% may be readily obtained. Acetic anhydride in RDX(*B*) liquors may be determined by direct titration with standard aniline–toluene solution at 0° C., using calcium hypochlorite as an external indicator. In routine analysis, a precision of ±2% may be obtained.

DETERMINATION OF NITRIC ACID

When equivalent amounts of nitric acid (0.3*M*) and sodium hydroxide (0.3*M*) were added to known volumes of aqueous acetic acid, it was observed that the dilution so effected caused a shift in the pH of the solution of approximately 0.01 unit for each 7 ml. water added. This observation was applied to the estimation of nitric acid in Bachmann RDX liquors as follows:

A 25.0 ml. sample was pipetted from the supernatant liquid of the RDX(*B*) slurry, and added to 100 ml. of water in a 250 ml. beaker. The solution was titrated with standard alkali (0.3*N*) using a suitable glass electrode assembly (Beckmann pH meter). Alkali was added until a pH of 2.07 was reached. The appropriate dilution correction of +0.01 pH units per 7.0 ml. alkali used was then added to the value 2.07, and the titration completed to this final pH. Determinations of known amounts of nitric acid added to RDX(*B*) filtrates could be made by this method with considerable precision, as indicated by the following typical results:

Original HNO_3, gm., in RDX(*B*) liquor	HNO_3 added, gm.	Total HNO_3 present, gm.	HNO_3 analysis, gm.
0.579	0.446	1.025	1.034
0.579	0.445	1.024	1.025
0.734	0.758	1.492	1.500

The concentrations of acetic anhydride and ammonium nitrate were varied over limits wider than those encountered in practice without affecting the precision of the nitric acid determination by more than 2 to 3%.

[1] *Manuscript received October 14, 1952.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Que., with financial assistance from the National Research Council.

DETERMINATION OF ACETIC ANHYDRIDE

Ivanov (1) has described a method for the estimation of aniline by a titration with acetic anhydride, using ground pulp paper as external indicator. The reverse of this, an estimation of acetic anhydride by titration with a toluene solution of aniline, has been mentioned by Zavarov (3). Preliminary experiments indicated that a similar method might be applicable to the analysis of acetic anhydride in RDX(*B*) liquors, but that a more satisfactory indicator than that of Ivanov was necessary. An adaptation of the calcium hypochlorite spot test for aniline (2) has been made for the purpose. The reagents and procedure were as follows:

Dry aniline (1.0 mole) is dissolved in dry toluene and made up to 1.0 liter. The indicator, a triturated mixture of calcium hypochlorite and water, is used externally.

A 20.0 ml. sample of Bachmann filtrate is pipetted into 25 ml. of toluene in a 250 ml. Erlenmeyer flask. This sample is kept in an ice-water bath during titration. Addition of toluene to the sample promotes the acetylation reaction. Aniline–toluene solution from a burette is added slowly, with continuous agitation of the flask. A precipitate of acetanilide is formed during the titration but does not interfere. The end point has been reached when a drop of titrated liquid in the flask produces an immediate intensely colored ring on the surface of the indicator, the color varying from reddish-orange to purple. A secondary orange color develops gradually, but should not be confused with the true end point. Near the end point of the titration, one minute should be allowed before testing for excess aniline in titrated material. This permits completion of the acetylation reaction between aniline and anhydride.

The aniline–toluene solution was standardized against weighed amounts of pure acetic anhydride dissolved in toluene, using the technique outlined above.

The method was checked under various conditions. Determination of known amounts of acetic anhydride added to RDX(*B*) liquors may be made with a precision of $\pm 2\%$, as shown by the following results.

Original Ac_2O in 20.0 ml. RDX(*B*) filtrate, gm.	Ac_2O added, gm.	Total Ac_2O, gm.	Ac_2O by analysis, gm.	% Error
3.331	1.000	4.331	4.251	−1.8
1.912	1.505	3.417	3.458	+1.2
2.041	2.860	4.901	4.846	−1.2
2.175	2.124	4.299	4.220	−1.8

Deviations of $\pm 2\%$ are also encountered in duplicate determinations of anhydride in RDX(*B*) liquors and represent the precision of the method.

There was a possibility of reaction between nitric acid present and the aniline added. Such a reaction would of course give high values for anhydride. Addition of 0.75 gm. of 98% nitric acid to a 20.0 ml. sample of RDX(*B*) liquor destroyed the specificity of the indicator for aniline. Since this effect did not exist if both nitric acid and ammonium nitrate in a molar ratio of 1:1

were added to a sample of normal RDX(*B*) mother liquor, it would appear that all the nitric acid in anhydrous RDX(*B*) liquor is present as a complex with ammonium nitrate or as acetyl nitrate. Experience has shown that if nitric acid is present in excessive amounts, as in a partially completed Bachmann reaction, the end point is difficult to establish. The limited solubility of ammonium nitrate in such reaction liquors may make it impossible to nullify the effect of the higher nitric acid concentration. However, with liquors corresponding to completed Bachmann reactions, no such interference has been encountered. It may be concluded that free nitric acid interferes with the indicator and that ammonium nitrate – nitric acid complexes do not. The method has the advantage that it measures only the acetic anhydride present in the Bachmann liquor.

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THERMAL NEUTRON CAPTURE CROSS-SECTION OF Na^{23} AND Mn^{55}[1]

By Rosalie M. Bartholomew, R. C. Hawkings, W. F. Merritt, and L. Yaffe[2]

ABSTRACT

The thermal neutron capture cross sections of Na^{23} and Mn^{55} have been determined using the activation method. The values are 0.53 ± 0.03 and 12.7 ± 0.3 barns respectively with respect to $\sigma_{Au}197 = 93$ barns. These agree well with recent pile oscillator results. The half-life for Mn^{56} is found to be 2.576 ± 0.002 hr.

A very complete survey of the thermal neutron capture cross section of a number of nuclides has recently been made by Pomerance (5) using the "pile oscillator" technique. If one compares this with results obtained by Seren, Friedlander, and Turkel (6) using the activation method, discrepancies are found in certain cases. If the pile oscillator value is higher than that obtained by the activation method, then a possible short-lived isomer of the nuclide in question may exist which the latter method has been unable to detect.

We have previously reported the re-determination of the capture cross section of Co^{59} (8) where some difference existed in the values obtained by the two methods. This paper deals with determination of the activation cross sections of Na^{23} and Mn^{55}. As a reference standard the cross section for Au^{197}, $\sigma = 93$ barns (3), at a neutron velocity of 2200 meters per second has been used in this work. This appears to be the best value if one makes sure that no contribution from the 5 ev. resonance in Au capture appears. Any change in the accepted value will of course cause a change in the experimentally determined cross sections as quoted here.

EXPERIMENTAL

(*a*) *Irradiation*

(*i*) *Sodium*

The sodium was irradiated as anhydrous 'spec-pure' sodium carbonate in a small silica capsule. The gold used was the highest quality obtainable (> 99.97%). In some cases gold leaf was used. Circular pieces were punched out of a sheet. The diameter of the circles was measured under a microscope. The area and weight of the original sheet were determined and thus the weight of gold used obtained. In other cases .001 in. thick gold foil was used and an amount weighed out such that the error in weighing was less than 1%.

The samples of sodium carbonate and gold were placed together in a polythene capsule so that they were very close together and received essentially the same neutron flux. Self-shielding of the gold was calculated to be of the order of 1% and appropriate corrections made.

The samples were irradiated in a 'self-serve' position where the neutron spectrum was predominantly thermal. Irradiations were also made with the entire sample

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[2] *Present address: Department of Chemistry, McGill University, Montreal, Quebec.*

wrapped in cadmium. The results of these latter irradiations enabled correction factors to be computed to allow for epicadmium capture (1.79% in the case of Au^{197} and 0.35% for Na^{23}). After irradiation the sodium carbonate was dissolved in dilute nitric acid and made to a standard volume. The gold was dissolved in a minimum amount of aqua regia, heated to prevent aggregate formation and made to a standard volume.

(*ii*) *Manganese*

The manganese was irradiated as 'spec-pure' manganese oxide (Mn_3O_4) sealed in a small silica tube along with the gold. The gold used was the .001 in. thickness as described in the previous section. Again the epicadmium contribution was determined. After irradiation the manganese oxide was dissolved in concentrated hydrochloric acid and made up to a standard volume. The gold was treated as described above. The epicadmium correction was 0.50%.

(*b*) *Measurements*

All measurements of disintegration rates were made using the 4π counter described by Borkowski (2) and Hawkings, Merritt, and Craven (4). Sources were deposited on thin (100 μgm. per cm.^{2}) zapon-formvar laminated films which had a coating of 25 μgm. per cm.^{2} gold sputtered on each side to make the film conducting.

The aliquots were removed from the solution using calibrated micropipettes and represented essentially weightless sources. Small corrections, of the order of one per cent, were made for absorption of the β-particles in the film (4) and for coincidence losses.

The disintegration rates were corrected for decay and lack of saturation of bombardment.

The decay corrections on Na^{24} were made using a half-life of 15.04 $\pm$ 0.06 hr. (7). The decay of Mn^{56} from the first two cross-section experiments was followed for 11 half-lives with a 4π proportional counter. The result of a least squares fit to the data is 2.576 hr. with a standard deviation of $\pm$ 0.002 hr. This figure, which is slightly less than the value of 2.586 $\pm$ 0.005 hr. given by Bishop, Wilson, and Halban (1), was used to make the decay corrections on Mn^{56}.

RESULTS

The results for the determination of the sodium cross-section are given in Table I.

TABLE I

Experiment No.	Corrected dis./sec./mgm.		Cross section of Na ($\times 10^{24}$ cm.^{2})
	Na $\times 10^{-7}$	Au $\times 10^{-8}$	
1	1.56	3.03	0.565
2	1.64	3.60	0.495
3	1.73	3.46	0.543
4	1.69	3.14	0.594
5	1.55	3.17	0.529
6	1.50	3.28	0.497
7	1.73	3.32	0.564
8	1.96	4.05	0.525
9	1.60	3.55	0.488
10	1.74	3.73	0.506
			Average 0.53 $\pm$ .03

The average obtained is 0.53 ± 0.03 barns where the error quoted is the standard deviation. The value obtained by Pomerance (5) was $0.47 \pm .02$ barns by the pile oscillator method, while the activation method used by Seren, Friedlander, and Turkel (6) gave 0.63 ± 0.19 barns.

The results for the manganese cross section are given in Table II.

TABLE II

Experiment No.	Corrected dis./sec./mgm.		Cross section of Mn ($\times 10^{24}$ cm.^{2})
	Mn $\times 10^{-8}$	Au $\times 10^{-8}$	
1	1.96	4.03	12.8
2	1.72	3.50	12.7
3	1.83	3.65	13.2
4	1.81	3.85	12.4
5	1.74	3.62	12.6
6	2.07	4.40	12.4
			Average 12.7 ± 0.3

The average obtained is 12.7 ± 0.3 barns where the error shown is the standard deviation. The value obtained by Pomerance was 12.8 ± 0.6 barns using the pile oscillator method, and 10.7 ± 3.3 barns by Seren, Friedlander, and Turkel using the activation method.

Our values for both the Na^{23} and Mn^{55} cross sections agree with the pile oscillator value and fall within the wide limits given by the activation value of Seren *et al.*

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STUDIES OF LIGNIN BIOSYNTHESIS USING ISOTOPIC CARBON

I. LONG-TERM EXPERIMENT WITH $C^{14}O_2$[1]

By J. E. Stone

ABSTRACT

Wheat plants were fed with $C^{14}O_2$ at the stage of growth corresponding to rapid lignification. Thereafter, plants were harvested every few days until maturity. The specific and total activity of the plants, and the specific and total activity in some lignin degradation products were determined. Results indicated that (i) $C^{14}O_2$ was still being respired from the plants at maturity, (ii) all the C^{14} which gets into the lignin does so within 24 hr. from the administering of $C^{14}O_2$, (iii) the total activity originally acquired by the syringaldehyde portion of the lignin remains constant throughout the growth of the plant, that is, the lignin (as represented by syringaldehyde) is a final end product of the plant and is not a part of the respiratory system, (iv) the total activity originally acquired by the vanillin suffers an initial drop for about two weeks after activation and then becomes constant, (v) the *p*-hydroxybenzaldehyde activity drops continuously throughout the life of the plant.

INTRODUCTION

When considering the mechanism of lignin biosynthesis it is usual to postulate a precursor which might by its structure and known reactions lead to lignin. There must be many intermediates between carbon dioxide and lignin but it is very probable that a simple aromatic compound, or to be more explicit, a phenol substituted in the para position, lies somewhere along the route. Klason (2) suggested that coniferyl alcohol or aldehyde might be a lignin precursor, and Freudenberg's recent work (1) on the polymerization of coniferyl alcohol to a lignin-like substance by polyphenol oxidase seems to support this contention.

It is probable that much light can be shed on this problem by the feeding of suspected precursors, labelled with C^{14}, to plants which are actively synthesizing lignin. Activity should appear in the lignin if the added compound is a precursor. One disadvantage to this method however is that carbon dioxide is the only precursor which can be added without risking some disturbance of the normal metabolism of the plant. A more serious difficulty arises owing to the fact that lignin is not a well-defined crystalline compound which can be obtained in a pure state. Because of this it would be rather difficult to decide whether activity which appeared in the isolated lignin was due to a true incorporation of the precursor into a normal lignin molecule or whether it was due to the copolymerization of the actual precursor and the supposed one which had been added. Polyphenol oxidases are present in many plants and the mere isolation of a radioactive lignin-like substance from a plant which had been fed a radioactive phenol would need interpreting with caution.

The difficulty goes even further than this because the lignin as isolated from the majority of plants is highly contaminated with complexes of the humic acid type which are definitely not lignin-like from the chemical point of view. Regardless of the precursor used, from $C^{14}O_2$ to C^{14} labelled coniferyl alcohol, the

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total activity acquired by the isolated "lignin" fraction would be meaningless.

It appeared necessary, therefore, to study the activity acquired by the crystalline degradation products of lignin, i.e. vanillin, syringaldehyde, and possibly *p*-hydroxybenzaldehyde, rather than lignin itself. It was decided to use $C^{14}O_2$ for activating the plants because, although it has the disadvantage of causing the majority of plant components to become radioactive after a few seconds, continuing growth in an atmosphere of normal carbon dioxide should distinguish between those which were intermediates and those which were stable end products. The former should become depleted in activity with time while the latter should increase in total activity.

It was hoped that the present work would provide answers to the following questions. After wheat plants are given a fixed dose of $C^{14}O_2$ and their growth is continued in carbon dioxide,

(1) How long does it take for the lignin, as represented by the three phenolic aldehydes, to acquire its maximum activity?

(2) Is lignin a stable end product of plant metabolism or is it part of a dynamic system with a consequent gradual loss of activity?

(3) What percentage of the activity originally administered to the plant shows up in the phenolic aldehydes? If an appreciable amount, this could be a convenient biological method for preparing C^{14} labelled aromatic compounds.

Future papers in this series will deal with the conversion of $C^{14}O_2$ to aldehydes during the first few minutes and hours of photosynthesis, and the feeding of multicarbon plant metabolites labelled with C^{14}.

EXPERIMENTAL

Apparatus

A dual purpose chamber was constructed for growing the plants in an enclosed atmosphere and for carrying out subsequent large sheet paper chromatography.

The dimensions of the chamber were 27 in. × 16 in. and 30 in. high, constructed of plate glass cemented into an angle iron frame and provided with casters for easy moving. The cover, also made of plate glass, was ground to fit the top of the chamber. This cover contained three ½ in. holes for (*a*) the carbon dioxide generator, (*b*) a thermometer, (*c*) a tube for watering the plants. When the chamber was used for chromatography these holes were used for adding the solvent to the three stainless steel troughs after equilibration of the paper. Air was drawn through a 2 in. hole in the bottom of the chamber and exhausted outside the greenhouse by means of a blower connected to a side hole. Illumination was provided by daylight supplemented by artificial light to give a total of 17 hr. of light and 7 hr. of darkness per day.

Procedure

Thatcher wheat was grown (three plants per 5 in. pot) under normal conditions until alkaline nitrobenzene oxidations indicated that the plants were reaching the stage when rapid lignification was about to set in (4). Nine pots containing 27 plants of similar size were selected and placed in the growing chamber. The latter was sealed and 1 millicurie of $C^{14}O_2$ added. Illumination was maintained for 24 hr. and then the blower started in order to sweep out any

remaining $C^{14}O_2$. After air had been drawn through the chamber and exhausted outside the greenhouse for one hour, a single plant was removed for analysis. Thereafter, a plant was removed at intervals of a few days up to the cessation of growth. As much as possible of the root was retained by carefully washing away the earth clinging to it.

The plants were dried in a rapid current of air at 35°C. and ground to pass a 40-mesh screen. Samples of about 10 mgm. weight were burnt to carbon dioxide in a normal combustion train and the carbon dioxide trapped as sodium carbonate and converted to barium carbonate. The barium carbonate was filtered and its "specific activity" at infinite thickness determined using a Simpson gas flow counting chamber and an RCL scaler. The residue remaining in the crucible after the combustion gives the percentage ash in the plant material and all results were calculated on an ash-free basis.

The nitrobenzene oxidations and determination of *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde were carried out by the micromethod of Stone and Blundell (3). About 60-80 mgm. samples were used and after the oxidation, one half of the liquor (0.5 ml.) was placed on a paper chromatogram sheet 18 in. × 22 in. and developed with the organic layer of petroleum ether – *n*-butyl ether – water 6:1:1. The pure phenolic aldehydes which separated on the paper were extracted with ethanol, the solutions evaporated to small volume (about 5 ml.), made alkaline with alcoholic potassium hydroxide, and aliquots removed for the determination of (*a*) percentage aldehyde, (*b*) specific activity of aldehyde. The latter was carried out by evaporating 1.0 ml. on to an aluminum disk and measuring the counts per minute obtained. The very small weight of material on each disk gave an "infinitely thin" sample so that the specific activity of the aldehydes recorded as counts per minute per mgm. should be interpreted on this basis.

RESULTS AND DISCUSSION

The results are shown graphically in Figs. 1 to 5 and are almost self-explanatory. Fig. 1 demonstrates the fall in specific activity of the total plant carbon

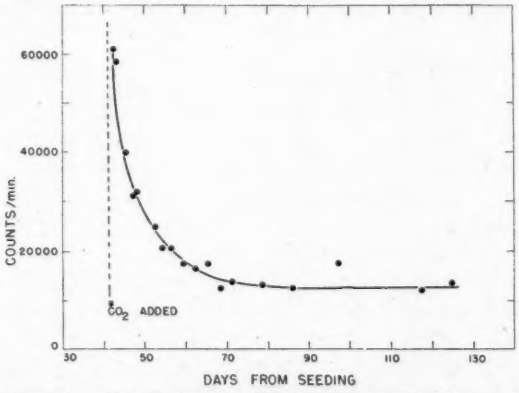


FIG. 1. Specific activity of total plant carbon (measured as barium carbonate, infinitely thick). $C^{14}O_2$ added on 41st day from seeding. First active plant removed for analysis 25 hr. later.

with time, this fall being due to two causes. One is the continuing growth of the plant in inactive carbon dioxide with a consequent dilution of activity. The other cause is the loss of $C^{14}O_2$ through respiration. This latter is demonstrated in Fig. 2

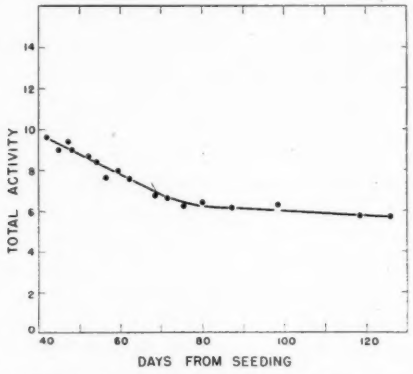


FIG. 2. Total activity of wheat plants. Weight (ash free) × specific activity.

where the total activity of the plant (specific activity × weight of plant) is seen to fall with time.

Figs. 3, 4, and 5 refer to the results of the alkaline nitrobenzene oxidation of the plants. Fig. 3 shows the change in the percentage of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde and indicates the age of the plants at the time of activation and at subsequent harvests. (In the other figures a number of points have been omitted for the sake of clarity.) It will be noted that the vanillin and syringaldehyde percentages are increasing quite rapidly during the course of the experiment, whereas *p*-hydroxybenzaldehyde rises only slightly.

The specific activities of the three aldehydes fall as the plant matures, this being demonstrated in Fig. 4. This fall is only to be expected since inactive lignin

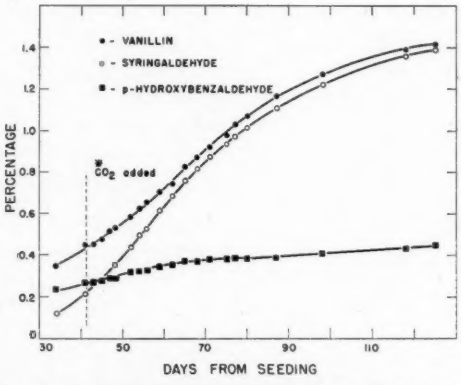


FIG. 3. Percentage of aldehydes based on whole plant.

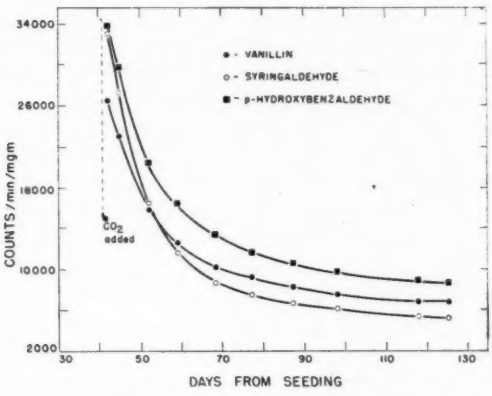


FIG. 4. Specific activity of aldehydes.

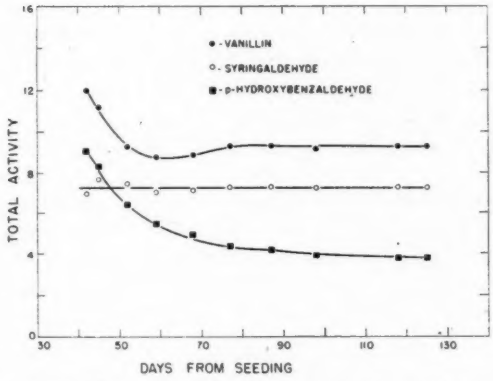


FIG. 5. Total activity of aldehydes (percentage × specific activity).

is being synthesized by the plant the whole time and is diluting the activity of the material formed immediately after the addition of $C^{14}O_2$.

Of more interest are the curves shown in Fig. 5 where the total activity of each aldehyde is given. The total activity is the specific activity (see Fig. 4) in counts per min. per mgm. multiplied by the percentage of aldehyde (see Fig. 3) and represents the total amount of activity acquired by that portion of the lignin molecule. The units of total activity are arbitrary ones and have not been calculated as microcuries since it is unnecessary for comparative purposes.

The curves of Fig. 5 are interesting in that each of the three aldehydes appears to behave differently. The activity of the *p*-hydroxybenzaldehyde drops continuously throughout the life of the plant and supports the contention put forward in a previous paper (4), that this aldehyde is derived from tyrosine present in plant protein and not from the lignin fraction of the plant. Tyrosine can lose active carbon in a number of ways as it is part of a dynamic system so that loss

of activity from its oxidation product, *p*-hydroxybenzaldehyde, is not surprising.

Syringaldehyde, on the other hand, is apparently derived from an irreversible system since it loses no activity over a period of two to three months and cannot therefore be involved in respiration unless its loss of activity is exactly compensated for by a gain in activity from other active plant components. This latter is very unlikely. It will also be noted that there is no gain in total activity after the first 25 hr. from administering $C^{14}O_2$ so that evidently the path from $C^{14}O_2$ to the parent substance of syringaldehyde is complete in less than this time. This implies that it would be necessary to study the changes taking place in the first 25 hr. in order to obtain information regarding intermediates in lignin formation, and the results of such a study will be reported in later papers of this series.

Considering the total activity in the vanillin fraction (Fig. 5) it is seen to be between *p*-hydroxybenzaldehyde and syringaldehyde in its behavior. An initial drop in total activity is followed by a levelling out to constant activity. The most probable explanation for this is that the young plants contain guaiacyl-type lignans which give rise to higher percentages of vanillin than does lignin. As the plant matures these lignans are polymerized or condensed in some way to form lignin which is less susceptible to oxidation. There is evidence (4) that young wheat plants contain guaiacyl-type lignans which disappear during the early stages of lignification so this explanation is not unlikely.

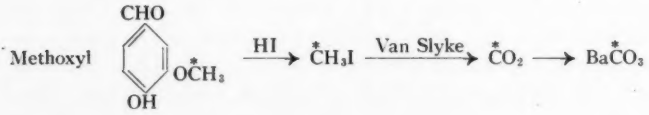
A separate experiment was conducted to find out what percentage of the activity which had been added to the plant appeared in the form of active aldehydes.

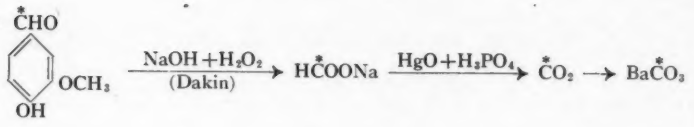
Five wheat plants (taken just prior to heading out) were kept in the dark for 18 hr. and then activated with 0.2 mc. of $C^{14}O_2$ in the light for a period of 24 hr. The plants weighed 6 gm. when dry and had absorbed 96% of the C^{14} added. The finely ground material was oxidized with alkaline nitrobenzene and the three aldehydes separated by chromatography. The results are shown in the table.

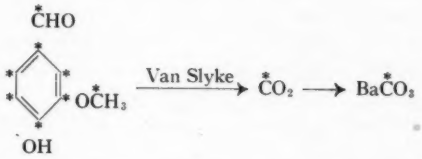
TABLE I

	Wt. of aldehyde, mgm.	Specific activity, μc. per gm. of carbon	Percentage of C^{14} added
p-Hydroxybenzaldehyde	10	60	0.3
Vanillin	49	40	1.0
Syringaldehyde	60	45	1.3

It would be expected that under the conditions of activation and growth used here, the carbon atoms in the compounds under consideration would have equal activity. This was shown to be the case. Taking vanillin as the example the reactions used for the degradations were:—



Aldehyde 

Total C 

It is possible that for certain purposes this could be a convenient method for obtaining C^{14} labelled phenolic aldehydes if the uniform labelling were not a disadvantage.

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STUDIES ON RDX AND RELATED COMPOUNDS

X. ANALYSIS FOR NITRIC ACID IN ACETIC ACID – ACETIC ANHYDRIDE MEDIA[1]

By R. A. Marcus[2] and C. A. Winkler

ABSTRACT

An analytical method has been developed for the estimation of nitric acid in acetic acid – acetic anhydride media, with a precision of 0.3%. The procedure involves the addition of a solution of potassium acetate in acetic acid to the sample. The excess is back-titrated conductometrically with a standard solution of nitric acid in acetic acid.

INTRODUCTION

A study of the formation of 1,9-diacetoxypentamethylene-2,4,6,8-tetranitramine (AcAn) from 2-acetoxymethyl-4,6,8-trinitrocyclotetramethylene tetramine (PHX), nitric acid, and acetic anhydride, the results of which are presented in a later paper, required a knowledge of the rate of nitric acid disappearance during the reaction. To this end, it was necessary to develop a method for analyzing nitric acid in an acetic acid – acetic anhydride medium, as outlined below.

EXPERIMENTAL

Potassium acetate dissolved in glacial acetic acid was added to the acetic acid – acetic anhydride reaction mixture containing the nitric acid to be analyzed. The addition was accompanied by precipitation of potassium nitrate, owing to its insolubility in the anhydride-medium. The mixture was then back titrated with a dilute acetic acid solution of nitric acid, and the titration followed conductometrically. During the course of the titration, potassium nitrate was again precipitated and a decrease in conductivity was observed.

When reaction of nitric acid with the excess alkali acetate was complete, further addition of reagent caused the conductivity to increase, unless the reagent

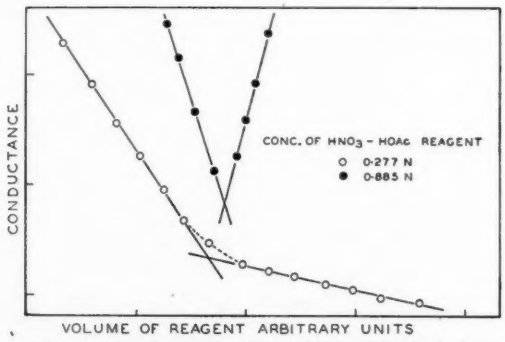

FIG. 1. Change of conductance with addition of analytical reagent.

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Que. with financial assistance from the National Research Council of Canada.

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was sufficiently dilute, in which case the conductivity continued to decrease but at a smaller rate than before. An example of both types of curves is given in Fig. 1.

Using the same acetic acid solutions of potassium acetate and nitric acid, several titrations of acetic acid–acetic anhydride mixtures containing known amounts of nitric acid, permitted a linear calibration curve to be established with the aid of which solutions of unknown nitric acid content could be analyzed with a precision better than 0.3%. This calibration method was adopted in preference to direct titration with potassium acetate – acetic acid solution for two reasons. First, unless there are a large number of nuclei of the nitrate present, the direct titration is very slow and tedious. Secondly, the addition of an excess of potassium acetate was effective in quenching immediately the nitrolysis reaction that was under investigation.

THE NONSPECIFIC PRECIPITATION OF PROTEINS BY POLYHAPTENIC DYES[1]

By J. L. Morrison

ABSTRACT

The serum proteins albumin and γ-globulin were precipitated by two polyhaptenic azobenzenearsonic acid dyes, at pH values in the region of or below the isoelectric points of the proteins. The occurrence of an optimum zone of precipitation suggests either a colloidal precipitation or the formation of a framework of dye and protein. The molecular ratios of dye to protein in the precipitate suggest increasing aggregation of the dye with lowering pH. The aggregation appears to be an acid constant effect. An estimate of the number of protein combining groups for one of the dyes is 22 for albumin, and 44 for γ-globulin. At pH values between 5.0 and 5.4, at which albumin is not precipitated, the precipitation of γ-globulin by the dyes is dramatically reduced by the presence of albumin. Apparently albumin forms soluble complexes with either the dye or γ-globulin or with both these substances.

In immunochemistry, the reactions between antisera and antigens are well characterized by their specificity (7). On the other hand, recently much study has been made of the nonspecific binding of simple substances by proteins (4, 5, 6). That nonspecific binding is a complicating factor in the reaction of simple antigens and antisera has been shown by Pardee and Pauling (9) who found that the reaction of polyhaptenic substances with purified hapten-homologous antibody is considerably different from that of the same substances with antiserum. They interpreted the differences as resulting from the combination of the polyhaptenic substances (which were azo dyes) with the serum albumin present in the antiserum, this combination serving to keep the dye monomer concentration low, and thus to prevent the formation of polymeric aggregates of dye molecules. It is well known that many dyes form aggregates in solution, and the formation of these aggregates has been verified for some of the dyes used in serological work (1, 10). Serum albumin is known to have a significant binding power for azo dyes (4, 5, 6).

The nonspecific binding of dyes and proteins is also of importance in connection with attempts to produce antibodies *in vitro* by controlled denaturation of serum protein in the presence of certain dyes (12).

Thus, experiments were carried out to discover nonspecific interactions between some polyhaptenic dyes used in serological reactions and some normal serum proteins. It is well known that these polyhaptenic dyes do not give precipitates with normal serum proteins in the usual pH range, around 8, of the serological precipitin reaction. However, by lowering the pH to around or below the isoelectric points of the serum proteins, the author found that precipitates could be obtained. Precipitation of proteins below their isoelectric points has been observed by Putnam and Neurath (15, 16) for horse serum albumin and the anionic detergent, sodium dodecylsulphate.

[1] *Manuscript received October 6, 1952.*
Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta. Presented at the Western Regional Conference, Chemical Institute of Canada, Saskatoon, Sask., October 25, 1952.

EXPERIMENTAL METHODS

Haptenic Compounds

The two polyhaptenic compounds used in the present investigation, 2-methyl-4,6-di(*p*-azobenzenearsonic acid)phenol (compound III) and 1,3-dihydroxy-2,4,6-tri(*p*-azobenzenearsonic acid)benzene (compound VI), were from preparations that have been previously described (13). Compound III had been shown by chromatographic tests to contain a small amount of an unidentified impurity. Solutions of the compounds were made in water and adjusted to pH 7 and to concentrations of about 0.57 and 0.80% respectively (in each case approximately 0.01 molar).

Some other haptenic substances used in single experiments are described later.

Proteins

The bovine γ-globulin that was used was kindly supplied by Armour and Co., with the label "Fraction II from bovine plasma". The serum albumin, provided through the courtesy of the Department of Physical Chemistry, Harvard Medical School, was labeled "Human albumin, run 35, fract. V, crystals". The proteins were dissolved in sodium acetate – acetic acid buffers between pH 3.72 and 5.72 and ionic strengths of 0.20 from pH 3.72 to 4.83 and 0.10 from pH 5.00 to 5.72, and were dialyzed against large volumes of these buffers before use.

Experimental Procedure

Either 0.5 or 1.0 ml. of protein solution was added to 0.5 or 1.0 ml. of each of a series of serially diluted dye solutions, the dilutions being made with buffer of the same pH and ionic strength as the protein solution. Protein concentrations were varied from 0.1 to 1.0%, so as to give a convenient amount of precipitate for analysis. The samples were immediately shaken by hand after mixing, and the sample tubes were stoppered with cork. The samples were refrigerated for two days and then were centrifuged. Then, without disturbing the precipitate a few milliliters of the same buffer were added to wash out extraneous solution, the tubes were centrifuged again, and were allowed to drain dry. The precipitate was dissolved in a few drops of 2*N* sodium carbonate solution and was analyzed with a Klett photoelectric colorimeter for the dye concentration, and with Nessler reagent for total nitrogen. It was found that the dyes did not yield their total nitrogen in a Nessler analysis; however, each dye gave a consistently constant percentage of its nitrogen in this analysis, 72.5 and 46% for compounds III and VI, respectively. In the calculations reported below the molecular weights of the proteins were taken to be 68,000 for serum albumin and 160,000 for γ-globulin.

EXPERIMENTAL RESULTS AND DISCUSSION

General Description of the Precipitations

It was found that each of the two dyes gave precipitates with each of the proteins in the lower part of the pH range investigated, the amount of precipitate falling off rapidly in the region of the isoelectric points of the protein (these are 4.8 for human serum albumin (3) and 6.5 for bovine γ-globulin (2)). Quan-

titative studies were made for each of the four systems γ-globulin – compound VI, albumin–compound VI, γ-globulin–compound III, and albumin–compound III, at 11 pH values nearly equally spaced in the range 3.72 to 5.72, in each case with the use of eight different amounts of dye differing by successive factors ½. A typical set of data is reported in Table I. Dye solutions of 0.5 ml. volume,

TABLE I

TYPICAL PRECIPITATION REACTION OF γ-GLOBULIN AND COMPOUND VI AT pH 4.62

Tube Number	Amount of dye added, mgm.	Composition of precipitate		
		Dye, mgm.	Protein, mgm.	Molar ratio of dye to protein
1	4.02	0.069	0.378	36.8
2	2.01	0.0756	0.387	39.3
3	1.00	0.0725	0.390	37.5
4	0.50	0.0597	0.342	35.1
5	0.25	0.0526	0.330	32.3
6	0.125	0.0392	0.237	33.3
7	0.062	0.0142	0.090	31.8
8	0.031	0.0074	0.042	35.4

containing varying amounts of compound VI in acetate buffer at pH 4.62 and ionic strength 0.20, were mixed with 0.5 ml. of similarly buffered γ-globulin containing 0.412 mgm. protein. The amount of compound VI varied from 4.02 mgm. to 0.031 mgm. by successive factors ½. The precipitates were analyzed for dye and protein. It is seen from the table that in the first three tubes over 90% of the protein was precipitated, and that the amount of precipitated protein then decreased. The molecular ratio of dye to protein in the precipitate remained essentially constant through the series, the average value being 35.2.

The change in ionic strength from 0.20 to 0.10 at pH 5.00 was necessary in order to secure precipitates above this pH. The dye–protein ratio of the γ-globulin – compound VI precipitate at pH 4.83 was determined at both ionic strengths and showed no difference.

With both compound III and compound VI, over 90% of the γ-globulin was precipitated in the optimum zone throughout the pH range 3.72 to 4.83. The amount precipitated then began to decrease, reaching about 50% at pH 5.25, 25% at 5.5, and 2% at 5.7. With both compounds the amount of albumin precipitated was over 90% of the amount present to pH 4.5, dropping to about 50% at 4.8, and about 20% at pH 5.0.

Colloidal Hypothesis

In general, in experiments at any one pH value, it was found that the amount of protein precipitated was a maximum in about the second or third tube – occasionally the fourth or fifth. The optimum zone of precipitation in the dye–protein systems appears to be similar to the equivalence zone of precipitation observed for a detergent–protein system (15, 16). Putnam and Neurath account for their results in terms of a mutual precipitation of oppositely charged colloidal particles, *as well as* a stoichiometric combination of detergent and protein.

However, certain important differences exist between the present dye–protein and the detergent–protein systems.

(*a*) Both the amount and composition of the dye–protein precipitates are dependent on pH. The optimum zone is not sharply defined (see Figs. 2-5). In the optimum zone, except for a few cases below pH 4.27, only part of the protein is precipitated by dye. In the γ-globulin systems in particular, for all dye concentrations at a pH greater than 4.27, more than 50% of the dye is still in solution (e.g. Table I). Thus, at a pH greater than 4.27, there is always both unprecipitated dye and protein in the supernatant liquid.

In contrast, both the amount and composition of the detergent–protein precipitates are independent of pH up to the isoelectric point of the protein. The equivalence zone is sharply defined. Practically all the detergent is in the precipitate below the region of detergent excess, and in the equivalence zone, all the protein is precipitated. Thus, within the equivalence zone, there is no detergent or protein in the supernatant liquid. To explain their observations, Putnam and Neurath suggest that the anionic detergent not only neutralizes the opposite net charge of the protein, but also combines with *all* the cationic groups in the protein, limited only by the amount of detergent present.

The ratios of dye to protein in their precipitates are largely dependent on pH for compound VI and on both pH and dye concentration for compound III. For example, in the γ-globulin – compound VI system, the dye–protein ratios for the precipitates are practically independent of dye concentration and completely dependent on pH at pH values above 4.27. In contrast, the ratio of detergent to protein in their precipitates is dependent on the ratio of substances before mixing, and independent of pH up to the isoelectric point of the protein.

(*b*) The dye–protein systems show a *decrease*, whereas the detergent–protein system shows an *increase* in precipitation with an increase in ionic strength. The latter is the expected behavior of a colloidal precipitation, whereas the former is similar to the behavior of a pure protein.

It would appear that the application of the colloidal hypothesis to account for the precipitations in the dye–protein systems is not as satisfactory as it is for the precipitations in the detergent–protein system.

Framework Hypothesis

The occurrence of a zone of optimum precipitation for the dye–protein system may also be compared with similar observations made for serological precipitations. To account for the latter, Pauling (11) and others have suggested a framework hypothesis. Without assuming the same ***kind*** of interaction as in specific serological reactions, the framework hypothesis may be applied to account for the present results. Then the decreased amount of precipitation in the dye-excess region can be explained by the formation of soluble complexes in which all the combining regions are saturated with separate dye molecules. The effect would be similar to the antigen-excess effect (solution of the specific precipitate in excess antigen). The decrease in the amount of precipitate in the low-dye region might be attributed simply to the presence of a smaller amount of dye. However, it is to be noticed in Table I (and this is typical) that the amount

of dye contained in the precipitate is only a fraction (about one-quarter) of the total amount of dye present, even in the last tube, in which only 1/10th of the protein is precipitated. This suggests that the dye remaining in solution is present in the form of small soluble complexes with protein molecules, and that this may be a phenomenon similar to that of solubility of a serological precipitate in excess of antibody, which is observed in some serological systems, and in particular those in which the precipitating antigen is a simple substance (13).

To further test the hypothesis that the precipitate is a framework of dye and protein molecules, some monohaptenic dyes and arsanilic acid were mixed with the proteins. Solutions of *p*-(*p*-azobenzenearsonic acid)phenol (pH 4.7), its 2-methyl derivative (pH 4.5) and a saturated solution of arsanilic acid were separately added to each of the two proteins (pH 4.45). The absence of precipitates in every case supports this application of the framework hypothesis.*

Dye Aggregation

Values of the molecular ratio of dye to protein in the precipitate as a function of pH are shown for the four systems in Fig. 1. Each point represents the average of the three largest values obtained with the eight tubes in a set. For either γ-globulin or albumin with compound VI the values in a set showed good consistency whereas the values for compound III were very sensitive to dye concentration, being larger for the higher concentrations.

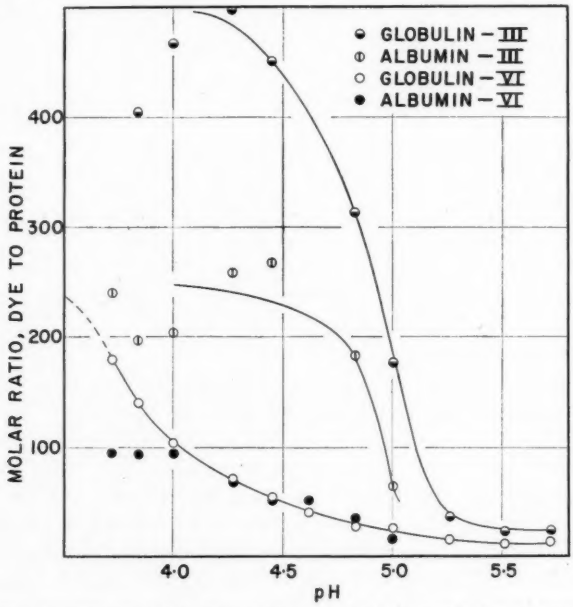

FIG. 1. The dependence of the molecular ratio of polyhaptenic dye to protein on pH.

**The referee suggests that this does not rule out the colloidal hypothesis, in that the monohaptenic dyes may be too small for aggregation. McMeekin (8) points out that sulphate and sulphonate detergent molecules containing more than eight carbon atoms are excellent protein precipitants.*

For γ-globulin and compound III the molecular ratio changes from a maximum of about 500 at low pH values to a lower asymptote of 22 for pH values 5.5 and 5.7. A similar course is shown by albumin and compound III, the values of the ratio being at each pH about 50% as great as for globulin and compound III.

The large increase in the dye–protein ratio with decreasing pH may be a result of either an increase in dye aggregation or an increase in the protein net charge or both these effects. It may be noted that in the case of the detergent–protein precipitations (15, 16) the net charge does *not* determine the amount of bound detergent. Its only role is to ensure that precipitation will occur. Thus, the bonding between the detergent and protein is not a net charge effect. Similarly in the present case, there are no theoretical grounds for assuming that the net charge of the protein should determine the amount of bound dye. Then, consider the case of γ-globulin and compound III at pH 4.62, in which the dye–protein ratio changes 13-fold in the first five tubes, yet, except for one tube, at least 40% of the dye remains in solution and in all five tubes more than 84% of the protein is precipitated. It is difficult to account for these facts by any means other than dye aggregation.

Thus, the *same* assumption as for the detergent–protein system is made, namely, that the protein combines with all the dye available up to a limit set by the number of dye-receptive groups in the protein. Since in all cases down to pH 4.27 and for most at a lower pH, there is some dye and protein in the supernatant liquid, it is considered that the increase in dye–protein ratio with decrease in pH is the result of an increase in the degree of aggregation of the dye molecules. It might well be expected that the tendency to aggregation would be small at high pH values because of the electrostatic repulsion of the negative ions, and would increase at low pH values as the benzene arsonate ions combine with hydrogen ions to form uncharged benzene arsonic acid groups. In other words, the dye aggregation may be an acid-constant effect.

The pH at half maximum for both curves for compound III is about 4.9; this value is close to pK_2 for compound III. The pK values of compound III were determined by titration, and found to be $pK_1 = 3.65$ and $pK_2 = 5.15$. The points for γ-globulin and compound VI lie on a smooth curve with asymptotic value 12 at pH values above 5.5, increasing to 180 at pH 3.7. The points for albumin and compound VI lie near those for γ-globulin and this compound but show a greater scatter. If the curve for γ-globulin and compound VI is assumed to be similar in form to those for compound III and to be approaching an asymptote of about 250, the characteristic pH value for compound VI can be estimated as 3.9. This value is close to pK_2 for this compound; by titration, it was found that $pK_1 = 3.26$, $pK_2 = 4.10$, and $pK_3 = 5.32$.

Protein Combining Groups

On the assumptions that the framework hypothesis applies, that at pH 5.5 to 5.7 the dye molecules combine with the proteins as monomeric molecules, and that the haptenic groups (two for compound III, three for compound VI) interact with separate combining groups of the proteins, the number of combining groups per γ-globulin molecule is calculated to be 44 from the asymptotic ratio 22 for compound III and 36 from the asymptotic ratio for compound VI.

There is some question, however, as to whether compound VI can use all three of its haptenic groups in combining with proteins; in specific serological precipitation compound VI has been found to have an effective valence of about 2.35, rather than 3, the decrease below 3 being attributed to steric hindrance (14). There are no independent determinations of the number of combining groups possessed by γ-globulin.

The curve for albumin with compound III in Fig. 1 is similar to that for γ-globulin for this compound, with the ordinate only about one-half as great. If the factor $\frac{1}{2}$ is assumed to hold into the region with larger pH, the asymptotic value with albumin would be 11, and, with consideration of the bivalence of this hapten, the corresponding number of combining groups would be 22. This value happens to coincide with the value found by Karush for the number of combining groups of bovine serum albumin with an azobenzoate ion (4).

Suppression of Precipitation by Albumin

It was found that at pH values of 5.0 and above, at which neither compound III nor compound VI produce a precipitate with albumin, the presence of albumin in the solution decreases the amount of precipitation of these compounds and γ-globulin. Measurements were made on a series in which the albumin concentration was varied and the pH kept at 5.26. The dye to protein molar ratios of the precipitates are given in Table II for globulin and for one of the

TABLE II

DYE–PROTEIN MOLAR RATIOS IN SOME ALBUMIN SUPPRESSION EXPERIMENTS AT pH 5.26*

Compd. III, mgm.	Compd. VI, mgm.	Molar ratio of dye to protein. γ-Globulin with:			
		Compd. III	Albumin and Compd. III	Compd. VI	Albumin and Compd. VI
2.79	4.02	118	89	17.5	22.2
1.39	2.01	68	47	16.9	15.4
0.69	1.00	36	28	15.8	15.1
0.35	0.50	22	28	15.9	12.5
0.175	0.25	22	55	14.8	21.0
0.085	0.125	25		10.1	
	0.062			5.4	

**In these experiments, 1 ml. dye solution containing 2.79 mgm. III or 4.02 mgm. VI was serially diluted with acetate buffer, and 0.5 ml. protein solution added, containing either 2.05 mgm. γ-globulin or 2.05 mgm. γ-globulin + 0.68 mgm. albumin. The protein solutions were buffered in acetate buffer of 0.10 ionic strength.*

globulin–albumin mixtures with each of the dyes. The amounts of precipitated protein for all the mixtures at pH 5.26 are shown in Figs. 2 and 3. Measurements of the suppression effect in which the protein concentrations were kept approximately constant and the pH varied from 5.00 to 5.40 are shown in Figs. 4 and 5. In all of the experiments 1 ml. dye solution and 0.5 ml. protein solution, at 0.10 ionic strength, were mixed.

The results may be comparable with the observation of Pardee and Pauling (9) at the somewhat higher pH of 6.2 in which serum albumin suppressed the precipitation of an antibody protein by its homologous polyhaptenic dye antigen.

No attempt was made to determine whether the protein in the precipitates

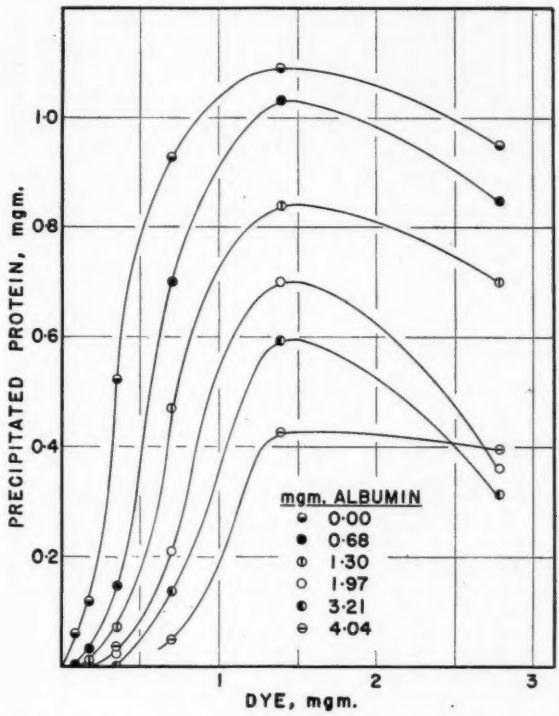

FIG. 2. The effect of albumin concentration on the precipitation of γ-globulin with compound III.

of the albumin suppression experiments was γ-globulin or a mixture of the two proteins. However, the dye–protein molar ratios of the precipitates of the systems dye–albumin–γ-globulin did not differ essentially from those for dye and γ-globulin alone (Table II), suggesting that the protein in the precipitates was probably γ-globulin. The reduction in the molar ratios of compound III in the presence of albumin, compared with its absence, is not inconsistent with the behavior of this compound. It has already been noted that the degree of aggregation of compound III, as indicated by the dye–protein molar ratios, depends on both pH and concentration, whereas the degree of aggregation of compound VI depends only on pH. In Table II, the smaller molar ratio in the presence of albumin could be a consequence of a reduction in dye concentration.

The results reported in this part are difficult to interpret because of the possible alternatives of the formation of soluble complexes of either dye–albumin, dye–albumin–globulin, or even albumin–globulin.

Nevertheless, one may speculate as to why the amount of precipitate formed in the presence of albumin is less than in its absence. It is seen in Figs. 2 to 5 that the effect of the presence of albumin is especially pronounced in the region where the amount of dye is small. This suggests that the effect is due to

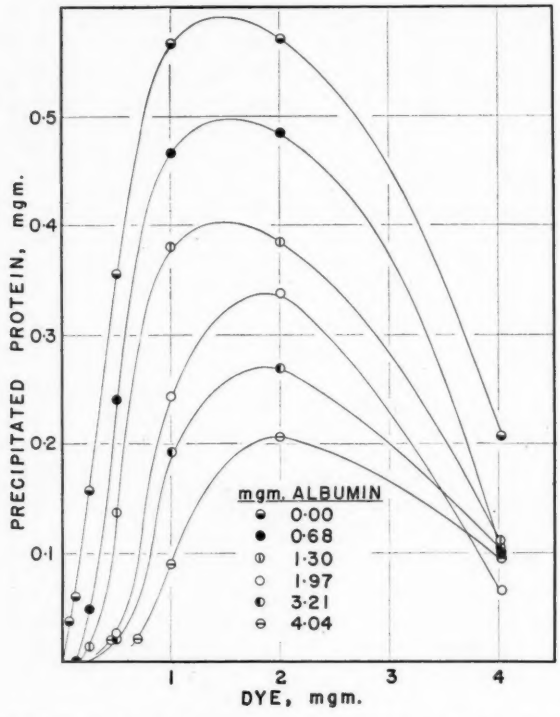

FIG. 3. The effect of albumin concentration on the precipitation of γ-globulin with compound VI.

the combination of the dye with albumin to form a soluble complex, kept in solution by the electrostatic repulsion of the strongly negatively charged molecules at these pH values, which are considerably greater than the isoelectric point of albumin. However, it is clear that the removal of the dye from solution by combination with albumin would alone not change the amount of precipitate at the maximum, but would only shift the position of the maximum. If, on the other hand, some globulin molecules are incorporated into soluble complexes with albumin, with the dye compound serving as a bond between them, this also would decrease the amount of precipitate. Inasmuch as at pH values greater than 5.0 and in the absence of albumin, only a part of the globulin is precipitated, some presumably being present as soluble complexes even at the optimum ratio of dye and protein, this explanation of the effect of albumin might be better given by saying that a larger amount of globulin is retained in solution in complexes because of the incorporation of albumin.

Production of Antibodies in Vitro

Pauling and Campbell (12) reported the production of antibodies *in vitro* by controlled denaturations of serum protein. *One* of their methods was to

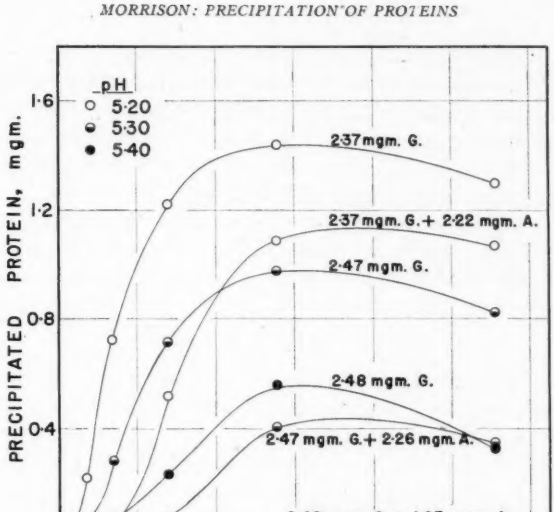

FIG. 4. The effect of pH on the suppression by albumin of the precipitation of γ-globulin with compound III.

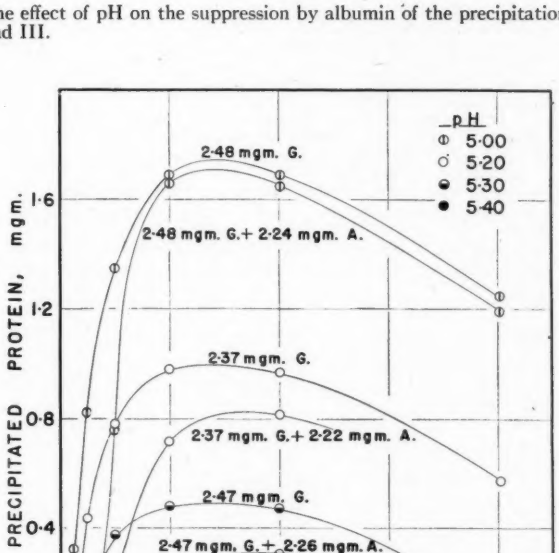

FIG. 5. The effect of pH on the suppression by albumin of the precipitation of γ-globulin with compound VI.

maintain γ-globulin at 57°C. in the presence of compound VI, for several days; then the dye was removed from the protein by dialysis. The modified protein was found to behave like natural antibody in forming specific precipitates with the same dye compound. Typical precipitin curves were formed, but at pH values of 5.0 and 5.5 which are well below the pH values of the specific precipitation of natural antibodies (pH 7 to 9).

The results in the present paper suggest that this particular method of producing antibodies *in vitro* would be difficult to validate because any specific precipitates would be masked by nonspecific precipitation of the protein by the same dye at the same pH values.

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KILIANI'S REDUCTION OF GLUCOSE AND FRUCTOSE CYANOHYDRINS TO THE CORRESPONDING HEPTANOIC ACIDS AND LACTONES[1]

By A. S. Perlin[2] and C. B. Purves

ABSTRACT

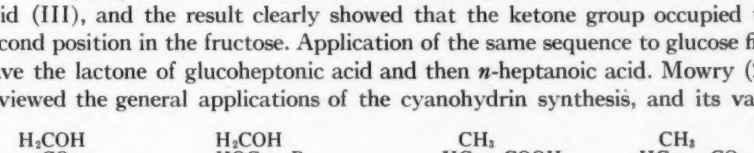
Kiliani's classic reduction of α-glucoheptonic (D-gluco-D-guloheptonic) lactone led to small, variable yields of *n*-heptanoic acid and varying amounts of the lactone of a hydroxy-*n*-heptanoic acid. A repetition of this work showed that the combined yields of these two products reached a maximum near 70% of theory when the reduction with constant-boiling hydriodic acid (b.p. 127° C.) and phosphorus was limited to two hours. Under these conditions up to 9/10ths of the product consisted of the lactone. After purification through the hydrazide, m.p. 89° C., the fragrant smelling lactone boiled at 61° to 62° C. at 10 mm. pressure, had a density of 0.9948, and a refractive index of n_D, 1.4405, both at 20° C. The supposition that this lactone referred to 4-hydroxy-*n*-heptanoic acid was confirmed by preparing it from the known 4-keto-*n*-heptanoic acid, which in turn was synthesized by an improved method.

The optimum time for reducing an uncrystallized mixture of α- and β-fructoheptonic acids under similar conditions was three to four hours, and the optimum yield of product was again near 70% of theory. This product consisted almost exclusively of the lactone of 2-methyl-4-hydroxyhexanoic acid. After purification through the crystalline hydrazide, m.p. 122° C., the lactone was recovered as a fragrant oil boiling at 48° to 49° C. at 2 mm. pressure, with density 0.9806 and refractive index n_D 1.4332 at 25° C. The structure of this lactone was not in doubt.

These lactones, prepared in good yield and with well defined hydrazides, were more readily characterized than the fully reduced heptanoic acids upon which Kiliani relied.

INTRODUCTION

While considering possible ways of locating carbonyl groups in oxystarches and oxycelluloses, it appeared that the method Kiliani used in 1885 to 1888 to solve the same problem for reducing sugars might be capable of development. In the case of fructose (structure I), for example, the addition of the elements of hydrogen cyanide gave the cyanohydrin which was hydrolyzed to the α,β-mixture of the corresponding fructoheptonic acids (II). Reduction of these acids with red phosphorus and hot hydriodic acid then yielded methyl-*n*-butyl acetic acid (III), and the result clearly showed that the ketone group occupied the second position in the fructose. Application of the same sequence to glucose first gave the lactone of glucoheptonic acid and then *n*-heptanoic acid. Mowry (29) reviewed the general applications of the cyanohydrin synthesis, and its value



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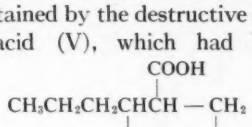
for the preparation of higher sugars was discussed in detail by Hudson (12); others (3, 13, 32) studied the preparation and hydrolysis of the sugar cyanohydrins, and the synthesis has been recently used to estimate carbonyl groups in hydrocelluloses and reducing sugars (11, 39), as well as to obtain sugars containing radioactive carbon (14). The reduction of the sugar acids to hydrocarbon acids, on the other hand, has received little attention since the time of Kiliani.

As early as 1860, Lautemann (24) employed concentrated hydriodic acid at 140° C., or "phosphorus di-iodide" and a little water near 100° C., to reduce lactic to propionic acid. Wislicenus (37) extended the work to pyruvic acid, and noted that the presence of phosphorus made possible a great economy in hydriodic acid. Benzylic acid was economically reduced to diphenylacetic acid in this way (26), and the general utility of the process was discussed by Miescher and Billeter (27). According to Kehrer and Tollens (15), the reduction of levulinic acid was preferably carried out for 12 hr. at 150° C. followed by eight hours at 200° C., but cyclic C_{10} to C_{15} hydrocarbons and tar were formed as by-products. The first application of the method to sugar acids was by Kiliani and Kleeman (23), who heated 1 part of gluconolactone under reflux with 10 parts of constant-boiling hydriodic acid and 0.4 parts of red phosphorus for seven hours. The relevant portion of the product, a red oil containing 2% of combined iodine, was de-iodinated with zinc and boiling hydrochloric acid, and the lactone of 4-hydroxyhexanoic acid (caprolactone) remained in a yield of 40% at most. This lactone gave caproic acid in small but unstated amount when the reduction was repeated at 160° instead of 127° C., but much of the starting material was recovered unchanged.

Kiliani's similar reduction of fructoheptonic acids (16) at reflux temperature gave about 60% of the theoretical yield of a heptanoic lactone, which was further reduced at 180°C. for 7.5 hr. to neutral substances and an unstated yield of an acid later proved to be 2-methyl hexanoic acid (17). The same acid was obtained in about 30% yield by Spoehr and Strain (36) who reduced a mixture of nonfermentable sugars, called "glutose", prepared by the action of alkaline phosphate solution on glucose or fructose. A temperature of 150° to 170° C. was employed for two hours in this reduction. Kiliani's experiments on the reduction of α-glucoheptonic (D-gluco-D-guloheptonic) lactone (18, 19) showed that only traces of *n*-heptanoic acid survived when similar temperatures were used, and that hydrocarbons were the major product. At reflux temperature, however, about half of the starting material was reduced to *n*-heptanoic acid in five hours, and more was recovered as the crude lactone of a hydroxy-*n*-heptanoic acid. The ready decomposition of the straight chain 7-carbon acid led to the adoption of reflux conditions in later reductions; galaheptonic acid after 1.5 hr. gave an unstated yield of heptonic lactone and an extremely small amount of a fatty acid (21); barium mannoheptonate yielded 15% of a lactone and 30% of *n*-heptanoic acid after five hours (8); and mannolactone (from L-arabinose) gave after five hours a large yield of caprolactone and *n*-hexanoic acid in smaller amount (20). Although the quantitative aspects of all the above work were unsatisfactory, they suggested that high and reproducible yields might be obtained if proper conditions were found for the reduction. The discovery of such conditions was the primary object of the present research.

Kiliani (19, 21) encountered difficulties in obtaining the *n*-heptanoic lactone in a pure condition, although the samples from the reduction of gluco- and galactoheptonic acids gave crystalline barium salts that seemed to be identical. This identity was extended by Fittig and Schmidt (10) to a barium salt corresponding to a lactone obtained by the destructive distillation, at 250° to 260° C., of propyl paraconic acid (V), which had been synthesized from bu-



V

tyraldehyde, sodium succinate, and acetic anhydride. The yield of lactone from this distillation was small (5%), and the major product was an unsaturated heptanoic acid, from which more of the lactone could be prepared by adding and then removing the elements of hydrogen bromide. Rupe, Ronus, and Lotz (31) also obtained the lactone by the action of sulphuric acid on a mixture of unsaturated acids prepared by heating α-bromoheptanoic acid with quinoline, and Bagard (1) by the destructive distillation of α-hydroxyheptanoic acid. In all three cases the carbon and hydrogen analyses were satisfactory. Since these observations hardly sufficed for the claim that the lactone was that of 4-hydroxyheptanoic acid, their reliability was incidentally confirmed by an independent synthesis during the present research. The lactone from fructoheptonic acid was certainly that of 2-methyl-4-hydroxyhexanoic acid, because Blaise and Luttringer (2) obtained it by reducing the corresponding 4-keto acid, which was synthesized from ethyl sodio-*n*-propionyl acetate and ethyl 2-bromopropionate. Another synthesis was carried out by Lukeš (25).

RESULTS AND DISCUSSION

Pure α-glucoheptonic lactone was heated under reflux for varying times with red phosphorus and constant-boiling hydriodic acid, and the resulting dark syrup was freed from combined iodine either by high pressure hydrogenation or, more conveniently, by nickel–aluminum alloy and caustic soda, according to Schwenck, Papa, and Ginsberg (34). Kiliani's use of zinc and hydrochloric acid for this purpose was found to be tedious and uncertain. After recovery, the product distilled almost completely between 97° and 113° C. at 20 mm. pressure as a pale yellow, acidic oil with a somewhat variable refractive index. This oil was accepted as the crude lactone of 4-hydroxyheptanoic acid, and the yields given in Table I were calculated on this basis.

When the de-iodinated products from the reduction period of 0.75 hr. were recovered in ether, a small amount of insoluble material remained. The reason that the yield in Experiment 2 exceeded that in Experiment 1 was perhaps because in the former case the above fraction was redissolved in alkali, reprecipitated by acid, and re-extracted with ether. This fraction was absent when the reduction was more prolonged. The table clearly shows that reduction for 1.75 to 2 hr. gave the highest yields of about 70% of theory (Experiments 3, 4, and 5) and that times of three and four hours were definitely less advantageous. Although the rate of the reduction was considerably lessened when the temperature was reduced from 127° to 100° C. (Experiment 9), a time much longer than 5.5

TABLE I

REDUCTION OF α-GLUCOHEPTONIC LACTONE[a]

Expt.	Hours	Yield,[b] %	Refractive index	
			n_D	°C.
1	0.75	54	1.4360	24
2	0.75	62	1.4355[c]	27
3	1.75	72	1.4360[d]	24
4	2	70	1.4365	25
5[e]	2	72	1.4370	25
6	3	60	1.4390	20
7[f]	3	53	(I)[g] 1.4390	25
			(II) 1.4390	25
8	4	47	(I) 1.4265[h]	19
			(II) 1.4400	19
9	5.5[k]	48	1.4380	24

[a] *Five grams of the lactone, 2 gm. of red phosphorus, and 70 gm. of hydriodic acid under reflux at 127° C.*

[b] *After de-iodination by hydrogenation and after a single distillation. Calcd. as % of the theoretical amount of heptonic lactone, 3.08 gm. from 5 gm. of the glucoheptonic lactone.*

[c] *Neutralization equivalent 128. Theory, 128.*

[d] *Neutralization equivalent 130.*

[e] *De-iodination with nickel–aluminum alloy.*

[f] *Double the quantities noted in (a) were used.*

[g] *Successive fractions of the single distillation denoted by (I) and (II).*

[h] *Neutralization equivalent, 202, 193. Theory 128.*

[k] *Reduction at 100° C. instead of 127° C.*

hr. might have given the highest over-all yield. A shorter period of 3.5 hr. at 100° C. gave too small a yield of de-iodinated product to warrant further investigation. The same result was encountered when the large excess of hydriodic acid employed in the reduction was replaced by a small amount of potassium iodide in presence of 90% phosphoric or 20% hydrochloric acid, and a considerable amount of charring occurred in the former mixture. Since redistillation of the crude lactone, even into a receiver cooled by solid carbon dioxide, occasioned a 10% loss, the yields in Table I were low by at least this amount.

In order to study the properties of the crude lactone in greater detail, that from Experiment 8 was fractionally distilled. The first fraction, roughly corresponding to (I) in Table I, boiled at 46° to 48° C. at 12 mm. pressure and had a low refractive index, n_D^{19}, 1.4130, and the high neutralization equivalent of 342, 351 (theory 128). These properties suggested that some decarboxylation occurred during the reduction, but the higher boiling fraction (b.p. 88° to 94° C.) was almost pure lactone. In Experiments 2 and 3, however, the neutralization equivalents were correct, and in these cases the somewhat low refractive indices pointed to contamination with heptanoic acid, n_D^{20}, 1.4234 (28). A 7% yield of this acid was isolated as the crystalline *p*-bromophenacyl derivative by oxidizing the crude lactone with nitric acid and distilling the liquor in steam. The crude lactone deposited Bagard's (1) "hydrazino-lactone" in high yield when warmed with hydrazine hydrate, and Darapsky and his collaborators (7) later showed that such compounds were true hydrazides. Hydrolysis of the pure, crystalline hydrazide, m.p. 89° C., made it possible to regenerate the pure lactone as a fragrant, colorless, neutral oil with a refractive index of n_D^{20}, 1.4405. In addition

to being rather stable to constant-boiling hydriodic acid under reflux, the lactone was recovered without change from an attempt to reduce it with Raney nickel catalyst and hydrogen at 110° C. and 2000 p.s.i. for 2.5 hr.

The early view that the lactone was derived from 4-hydroxyheptanoic acid was supported by the recovery of succinic acid in 35% yield from a sample oxidized with boiling nitric acid. Confirmation was obtained when the hydrazide of the lactone from the Kiliani reduction failed to depress the melting point of the hydrazide of the hydroxy acid prepared by reducing crystalline 4-keto-*n*-heptanoic acid with aluminum isopropylate, or by hydrogenation over a platinum oxide catalyst. Bouveault and Bongert (4) synthesized the keto acid by a standard method that established its structure, but they failed to record a yield. The sample used on the present occasion was obtained in 27% yield by condensing succinic anhydride with di-*n*-propyl cadmium in isopropyl ether, or by the general method of Cason and Prout (6).

Since the separation and crystallization of the isomeric α- and β-fructoheptonic lactones was difficult and gave poor yields (22, 33), the reductions with hydriodic acid and red phosphorus were carried out on the crude α,β-mixture obtained when the cyanohydration of fructose, and the subsequent hydrolysis of the cyanohydrin, were known to be at least 96% complete. After the reductions at 127° C., which were standardized except for time, the products were freed of combined iodine and once distilled as already described. The colorless, neutral oils boiled at 50° to 55° C. and 1 mm. pressure and were more uniform as regards refractive index than those from α-glucoheptonic lactone. When condensed with hydrazine hydrate, they yielded the crystalline hydrazide, melting at 123° to 124° C., characteristic of the hydrazide of 2-methyl-4-hydroxyhexanoic acid (IV) (2). Acid hydrolysis of the purified hydrazide then gave the pure lactone as a fragrant oil. The yield of this oil in the crude state was not very sensitive to the time of reduction over the period two to five hours (Table II) but the optimum was probably near four hours.

Although fructoheptonic was somewhat less easily reduced than α-glucoheptonic acid to the lactone of the corresponding 4-hydroxy fatty acid, in both cases the crude yield was at least 70%, and the lactones were readily characterized as crystalline hydrazides. Kiliani's further reduction of these lactones, in much poorer yield, to the unsubstituted fatty acids therefore appeared to be unnecessary in a modern repetition of his research.

EXPERIMENTAL

All melting points were uncorrected.

Reduction of α-Glucoheptonic Lactone

Following Kiliani's (18) method, 5 gm. samples of the pure, crystalline lactone (13) were heated under reflux with 70 gm. of constant-boiling hydriodic acid (sp. gr. 1.7) and 1.7 to 2.0 gm. of red phosphorus. In other experiments which used potassium iodide in place of the hydriodic acid (26, 27), 5 gm. of the lactone was heated under reflux with 35 cc. of 90% phosphoric acid, 3 gm. of red phosphorus, and 1 gm. of potassium iodide; or with 50 cc. of 20% hydrochloric acid, 5 gm. of red phosphorus, and 3.5 gm. of potassium iodide. After the heating

period, which varied from 45 min. to six hours, the reaction mixture was cooled, diluted with 150 cc. to 200 cc. of water, and extracted continuously with ether for 12 hr. A more rapid procedure was to saturate the liquor with ammonium sulphate and to extract three or four times with ether in a separatory funnel. The ether extract was filtered through glass wool to remove any phosphorus and was carefully concentrated at atmospheric pressure to a syrup.

In the earlier runs the removal of combined iodine from this syrup was accomplished by solution in 110 cc. of *N* sodium hydroxide and hydrogenation for two hours with hydrogen at 2000 p.s.i. and 110° C. over 2 gm. of Raney nickel catalyst (30). The liquid, originally turbid, changed to a clear yellow solution from which nickel was removed by filtration; acidification to pH 1 with 10% sulphuric acid followed, and then continuous extraction for three hours with ether. An alternative, and more convenient, method of de-iodination (34) consisted of dissolving the syrup in 80 cc. of 2 *N* sodium hydroxide contained in a tall, narrow, 500 cc. beaker covered with a watch glass. Six grams of Raney nickel–aluminum alloy powder was gradually and cautiously added during 10 min., and after the foaming subsided the mixture was kept at 90° to 95° C. for about 75 min. with occasional stirring. Sulphuric acid, 10%, was then added until the aluminum hydroxide initially precipitated was redissolved, and the extraction with ether was carried out for three hours.

The residue from the dried ether extract gave a pale yellow oil when distilled, preferably at 20 mm. pressure and into a receiver cooled in acetone – solid carbon dioxide; b.p. 109° to 113° C. (20 mm.), 88° to 94° C. (11 mm.), and 68° to 70° C. (1 mm.). Yields were recorded in Table I. Although the still-pot retained traces of charred material, decomposition caused by any entrained sulphuric acid probably did not occur during the distillation, because charring was not increased by the prior addition of a few milligrams of this acid. Kiliani's conclusion that the oil was impure heptonic lactone was supported by the low (0.8%) and variable results of combustion analyses.

Hydrazide of 4-Hydroxy-n-heptanoic Acid

The crude lactone, 0.5 gm., was heated upon the steam bath for two hours with 0.2 gm. of hydrazine hydrate (2). Upon cooling, the liquid solidified and the mass was twice recrystallized from ethyl acetate. The light white crystals (0.2 gm.) melted correctly at 88° to 89° C. (1) and a mixed melting point with an authentic sample (see below) was not depressed.

Lactone of 4-Hydroxy-n-heptanoic Acid

A 0.8 gm. sample of the pure hydrazide, dissolved in a small amount of water, was made strongly acid with dilute sulphuric acid and was heated for two to three minutes on the steam bath. The ether extract was filtered through glass wool, was dried, and the residue was distilled through a semimicro Cooke–Bower column (5). Three fractions were collected at 61° to 62° C. and 2 mm. pressure, and a fourth at 62° to 67° C., the total yield being 0.4 gm. The colorless oil had a density d_{20}^{20}, 0.9948, a refractive index, n_D^{20}, 1.4405, was neutral toward aqueous sodium carbonate, and gave a negative test for the hydroxyl group with phenyl and α-naphthyl isocyanate. Calcd. for a lactone $C_7H_{12}O_2$: mol. refraction, M_D,

34.08; neutralization equivalent, 128. Found: M_D, 33.92, neutralization equivalent, 128. Molecular refractions were calculated as described in the book by Shriner and Fuson (35), and neutralization equivalents after saponifying 15 to 20 mgm. samples in aqueous ethanolic alkali by warming for 15 to 20 min. on the steam bath.

Oxidations of 4-Hydroxy-n-heptanoic Lactone

The procedure used by Fittig and Messerschmidt (9) for valerolactone was followed.

(*a*). A pure sample, 2.3 gm. from a Kiliani reduction, was heated under reflux with 82 cc. of aqueous nitric acid (1 vol. : 1 vol.) for one hour, after which time the evolution of oxides of nitrogen had almost ceased. The liquid was then steam-distilled into a receiver cooled to − 10° C., but ether extracted from the 400 cc. of distillate no acid that gave a crystalline *p*-bromophenacyl derivative. Nevertheless, propionic, butyric, and acetic acid (from malonic acid) were probably present. Concentration of the still residue *in vacuo* yielded 0.74 gm. (35%) of succinic acid melting at 165° to 171° C., and at 170° to 175° C. when mixed with an authentic sample, m.p. 185° C. Recrystallization raised the m.p. to 180° C., and the mixed m.p. to 181° to 183° C.

(*b*). The once distilled, but still crude, acidic lactone from a Kiliani reduction, 1.53 gm., was oxidized with nitric acid, 54 cc., and the liquor steam distilled as just described. The distillate, 200 cc., was strongly acidified with sulphuric acid and was extracted four times with ether. Distilled water, 3 to 4 cc., was added to the extract and after evaporation of the ether the aqueous residue was nearly but not quite neutralized with dilute sodium hydroxide. The solution was then heated under reflux with 1 gm. of *p*-bromophenacyl bromide and 10 cc. of ethanol for one hour (35), and the crystals deposited when the solution cooled were twice recrystallized from 50% ethanol. The first crop, 0.18 gm., melted at 70° to 71° C., unchanged by admixture with the *p*-bromophenacyl derivative of authentic *n*-heptanoic acid (28). The melting point of the second crop, 0.09 gm., was increased from 63° to 65° C. to 68° to 70° C. by a similar admixture. These yields corresponded to one of 0.11 gm. of free heptanoic acid, or to about 7% of the original crude lactone.

Improved Synthesis of 4-Hydroxy-n-heptanoic Lactone

Di-*n*-propyl cadmium, when prepared by a general procedure (6), proved too volatile to be readily isolated from the ether–benzene used as a solvent. Magnesium turnings, 8.8 gm. (0.37 mole), were suspended in 200 cc. of anhydrous, peroxide-free isopropyl ether and 29 gm. (0.37 mole) of dry 1-chloropropane was slowly added under an atmosphere of nitrogen. After gentle heating to dissolve the magnesium completely, the solution was cooled in an ice bath and 34.8 gm. (0.19 mole) of anhydrous cadmium chloride was added in one portion. Thirty grams (0.3 mole) of pure succinic anhydride was added in one portion, the mixture was heated under reflux for four hours and was kept at room temperature for a further 12 hr. before being slowly poured into an excess of ice and dilute sulphuric acid. The aqueous phase was extracted with isopropyl ether and the extract combined with the upper layer. Acids were removed from the upper layer by

four extractions with 5% sodium carbonate, and were recovered by extracting the acidified sodium carbonate solution for 12 hr. with ether.

The residue from the ether, 21.7 gm. of a yellow oil, when distilled at 1 mm. pressure yielded 12.5 gm. (27%) of a clear colorless oil that boiled at 95° to 105° C. and crystallized readily in the receiver. Two recrystallizations raised the melting point to 48.5° to 49.5° C., in agreement with that quoted for *n*-4-ketoheptanoic acid (4, 25). The analyses and neutralization equivalent were also correct. In one experiment employing 15 gm. of succinic anhydride, the ether extract yielded 6.6 gm. of succinic acid in addition to the main product.

One gram of the pure keto acid was reduced by boiling under gentle reflux for 90 min. with 1 gm. of aluminum isopropoxide and 7 cc. of toluene, according to the procedure of Young and co-workers (38). Toluene was then allowed to distill slowly from the solution through a Widmer column, and was replaced at intervals by fresh toluene until the distillate amounted to 20 cc. three hours later. A slurry made from 30 cc. of 6 *N* sulphuric acid and crushed ice was added to the still residue, and the mixture was extracted six times with ethyl ether until the gel originally present had disappeared. Distillation of the oil recovered from the ether–toluene yielded 0.64 gm. (65%) of 4-hydroxy-*n*-heptanoic lactone with the correct neutralization equivalent and refractive index. The hydrazide melted correctly at 88° to 89° C. (1). As others observed (31), the lactone had a strong odor reminiscent of clover.

Reduction of α,β-Fructoheptonic Acid

Five grams of pure fructose, dissolved in 75 cc. of water, was mixed with 25 cc. of a solution containing 2.4 gm. of sodium cyanide (75% excess) and buffered to pH 9.5 with acetic acid. A preliminary experiment showed that the condensation was about 96% complete after 60 hr. at room temperature. At the end of this time, the mixture was diluted to 300 cc. and 150 cc. were distilled *in vacuo*. The still residue was again diluted to 300 cc. and the distillation repeated in order to complete the hydrolysis of the cyanohydrin and the expulsion of the ammonia. After acidification to pH 3 with hydrochloric acid, the still-residue was evaporated *in vacuo* to expel hydrogen cyanide and to recover the sodium salts of α- and β-fructoheptonic acid as a thick, yellow-brown syrup contaminated with sodium chloride.

A mixture of the syrup with 55 cc. of constant-boiling hydriodic acid was gently warmed until complete solution occurred without charring, and 2.5 gm. of red phosphorus was then added. The flask was connected to a small Liebig condenser and a slow distillation was carried out until the vapor temperature reached 125° to 127°C., or that of constant-boiling hydriodic acid; thereafter heating was continued under gentle reflux for two to five hours. At the end of this period the distillate was combined with the reaction mixture, and the products were isolated by extraction with ether followed by de-iodination with nickel–aluminum alloy, as already described for the reduction of glucoheptonic lactone. The final product, when distilled at 50° to 55° C. and 1 mm. pressure, was a clear, colorless oil with a refractive index near n_D^{25} 1.4330. Yields were recorded in Table II.

Condensation of this oil with hydrazine yielded a hydrazide whose nitrogen

TABLE II

REDUCTION OF THE α,β-FRUCTOHEPTONIC ACID MIXTURE[a]

Expt.	Hours	Yield[b], %	Refractive index	
			n_D	°C.
1	2	59	1.4340	25[c]
2	2	57	1.4335	27[d]
3	3	62	1.4340	24
4	4	72	1.4320	25
5	4	64	1.4345	25
6	4	74	1.4330	24
8	5	68	1.4320	28

[a] *Five grams of fructose converted to the heptonic acids and reduced with 2.5 gm. of red phosphorus and 55 cc. of constant-boiling hydriodic acid at 127°C.*

[b] *Based on the theoretical yield, 3.55 gm., of 2-methyl-4-hydroxyhexanoic lactone from 5 gm. of fructose.*

[c] *Neutralization equivalent 128. Theory 128.*

[d] *Neutralization equivalent 129.*

content of 17.5% and melting point of 123° to 124° C., after two recrystallizations from chloroform, were those of the hydrazide of 2-methyl-4-hydroxy-4-ethyl butyric acid (2). The hydrazide was hydrolyzed with sulphuric acid as previously described and the product was distilled at 2 mm. pressure in the Cooke–Bower (5) column. Three fractions were collected at 48° to 49° C. and a fourth at 48° to 54° C. The product, 2-methyl-4-ethyl butyrolactone (structure IV), was a colorless oil with a delicate odor, with n_D^{25} 1.4332 and a density, d_{20}^{25}, 0.9806. Found: neut. equiv. 127, 128; M_D 33.94. Calcd. for a lactone $C_7H_{12}O_2$: neut. equiv. 128; M_D 33.99.

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AN ELECTRON-MICROGRAPH STUDY OF OXIDE FILMS ON ELECTROPOLISHED SURFACES OF IRON[1]

By E. J. Caule and M. Cohen

ABSTRACT

An electron micrographic study has been made of iron surfaces subjected to electropolishing. It has been found that although electropolishing produces a relatively smooth surface, the iron is covered with a film. The strengthening of this film by oxidation and its disruption by reduction with hydrogen have been followed. The film is assumed to be oxide and its thickness has been estimated to be approximately 100 Angstrom units. The effect of reduction of the film on surface area and thus on initial oxidation rates of the reduced specimen has been considered.

INTRODUCTION

For some time electropolishing has been advocated as a method of preparation of metal surfaces for studies of oxidation rates. This has arisen from the fact that metallographic grinding and polishing produces a surface which is distorted and is not representative of the metal. It has also been shown (4, 14) that in some cases oxide films and inclusions are formed by mechanical polishing.

For most common metals and alloys, the proper electropolish treatment, once found by trial and error, will give a high degree of polish. This is assumed to be associated with a low specific area (or low "roughness factor"), a desirable feature for quantitative oxidation study since a surface of low specific area will be more invariable, as oxidation proceeds, than one of high specific area. In discussing the cleanliness and lack of disturbance in the structures of electropolished surfaces, Jacquet (7) showed that electrodeposits of copper on electropolished copper electrodes will follow the crystal pattern of the electrode, while on mechanically polished electrodes they will set up their own grain structure and orientation. His conclusion that electropolishing gives a clean surface was supported by Allen (1) who by two independent methods found no oxide on electropolished copper. On the other hand, Raether (12) used electron diffraction on copper and aluminum surfaces and concluded that there was a film produced by electropolishing which affected the rate of subsequent oxidation of the specimens in air. Hoar and Farthing (6) by an indirect method detected a film on brass while it was being electropolished. Cohen (3) has detected an oxide film on electropolished iron by the use of electron diffraction. Wilms (15) has produced micrographic evidence for the existence of a film on electropolished aluminum. Furthermore, it will be noticed in any collection of technical or scientific formulae for electropolishing baths that a great number of such successful formulae contain oxidizing agents, of which the most common is perchloric acid. There is therefore sufficient evidence to suspect that electropolished metal surfaces may be covered by very thin films whose thickness may be controlled by variables not yet appreciated.

The work to be described below is a study, with the aid of the electron microscope, of the topography of the surfaces produced by the electropolishing of one

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particular batch of iron in one successful bath liquid. Since it was not a comparative study of formulae or metals, care must be exercised in drawing general conclusions from the results. Gulbransen and his co-workers (10) have employed the electron microscope in oxidation experiments to examine oxide films which had been stripped from the substrate metal; since these were examined by transmission they could give little information about the topography of the surfaces. The same is true of the work of Mahla and Nielsen (8). In the present research plastic replicas of iron surfaces were made and shadow cast with chromium. Changes in surface structure due to oxidation and reduction were used to determine the normal features of the electropolished metal surface.

EXPERIMENTAL

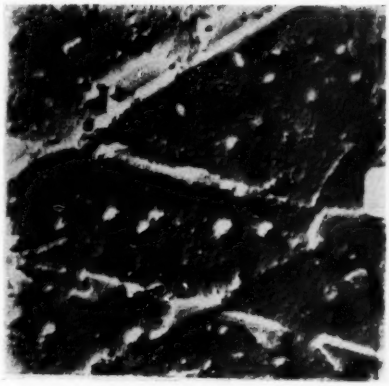
The iron used was a sample obtained through Dr. U. R. Evans of Cambridge University from the British Iron and Steel Research Association. Its analysis was: C—0.005, Si—0.0075, S—0.013, P—0.003, Mn—0.007, Ni—0.018, Cr—0.02, Cu—0.003, O—0.12, N—0.008, H—0.00009%. As supplied, it was in sheets 15 cm. × 30 cm. × 0.003 cm. From these sheets specimens were cut to a size of 1 cm. × 1½ cm. and were degreased in benzene. A soft iron wire was spotwelded to the middle of one edge to provide a lead for the electropolishing current.

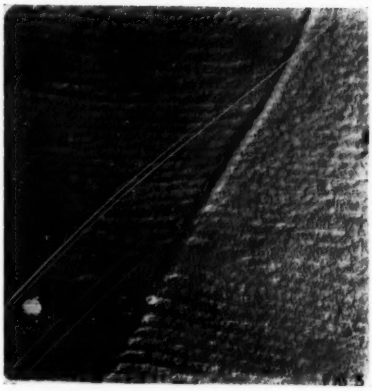
The conditions necessary for electropolishing this particular type of iron were determined by trial and error. The composition of the electropolish bath is based on that used by Radanovich and Wert (11), i.e., 850 cc. ethyl alcohol, 70 cc. water, and 50 cc. concentrated perchloric acid, but was modified by the addition of 23 cc. concentrated hydrochloric acid. The current density used was 1.0 ampere per sq. cm. and the temperature of the bath was kept below 18° C. by surrounding the bath with copper cooling coils. After having been polished for 30 sec. the specimen was rapidly washed in distilled water, then in alcohol, then in distilled water, and finally again in alcohol, after which it was allowed to dry. Drying was hastened by waving the specimen in the air.

The replicas of the metal surface were made as follows. A drop of a solution of ½% Formvar in 1,4-dioxane was placed on the specimen and the excess drained off the lower edge onto filter paper. The specimen was then held at a 45° angle until the solvent had evaporated, usually in three minutes. It was heavily scored with a razor blade in squares about 3 mm. on an edge. Double-sided "Scotch tape" was pressed down on the plastic after the plastic had been moistened by heavily breathing on it and the tape was then removed smoothly from the metal surface. The replica, now on the "Scotch tape", was shadow cast with chromium at an angle whose tangent was ⅕, after which it was removed from the tape by gentle agitation in amyl acetate. Sections were picked up on portions of 250 mesh screen, washed in fresh amyl acetate, and finally picked up on the 200 mesh grids used in the electron microscope. The instrument used in obtaining the pictures was an R.C.A. model EMU.

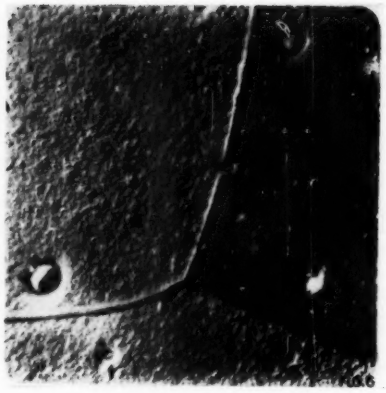
Oxidations and reductions were performed in the infrared furnace previously described (2). Specimens to be treated were spotwelded at one corner to a chromel–alumel thermocouple and supported in the furnace. They were then heated in a vacuum after which appropriate pressures of air or of hydrogen were

PLATE I





1. Mechanical polish. ×16,000
2. Electropolished. ×6,000
3. Electropolished, reduced in 0.05 mm. H_2 at 360°C. for 20 min. ×6,000
4. Electropolished, reduced in 0.05 mm. H_2 at 440°C. for 45 min. ×8,000



5. Same specimen as 4. ×11,000
6. Electropolished, oxidized in 0.01 mm. air at 310°C. for 0.5 minute to purple brown color. ×6,000
7. Electropolished, oxidized to blue temper color. ×11,000
8. Electropolished, reduced in 0.05 mm. H_2 at 550°C. for 62 min., then oxidized in 0.01 mm. air at 250°C. for 1 min. ×16,000

admitted. As mentioned in the description of the furnace, good control of the duration of heating was possible. After removal from the furnace, the test pieces were covered with plastic solution within a few minutes.

RESULTS

Electron micrographs were made of replicas of specimens which had been treated as follows:

(*a*) As electropolished.
(*b*) Reduced after electropolishing.
(*c*) Oxidized after electropolishing.
(*d*) Reduced and re-oxidized after electropolishing.

For comparison, a specimen which had been given a metallographic mechanical polish is shown. The exact treatments are given in the captions of the photographs. The photographs are "reverse prints" and have the same appearance as the negatives.

Photograph 1 is typical of a mechanically polished specimen, while photograph 2 is typical of an electropolished specimen and it is evident that the electropolishing treatment produces a relatively smooth surface. Photographs 3, 4, and 5 are of electropolished specimens which have been reduced in hydrogen, with 4 and 5 having been reduced at a higher temperature for a longer time. The reduction in hydrogen leads to a furrowed surface with patches of film and some distortion of the surface lifting the film. There is also a decrease in the sharpness and depth of the boundary. Photographs 6 and 7 are of electropolished surfaces which have been oxidized to a purple brown and a blue color respectively. The main features of the pictures are the change in texture on oxidation and the filling in of the grain boundaries with increasing oxidation. Photograph 8 is of an originally electropolished surface which was reduced and then oxidized to a light golden color. This treatment results in a very rough surface, which, according to Evans (5) is necessary for the production of temper colors. A close examination shows traces of what appears to be an old boundary.

DISCUSSION

A comparison of the electronographs of the mechanically polished and electropolished surfaces of iron (Figs. 1 and 2) shows that the electropolished surface is much the smoother. This has also been observed with electron micrographs of copper (13).

On treatment of an electropolished surface with hydrogen there is a shrinking and breaking effect on it. This film is most probably an oxide film. Examination of photographs 3, 4, and 5 shows that light reduction furrows the surface and that further reduction results in small patches of oxide being left as islands on a reduced surface. In some cases, as in photograph 4, there is a comparatively large area of unreduced oxide (in the center of the photograph) with probably some curling at the edges. The shadowing on photographs 4 and 5 is sufficiently sharp to allow an estimate to be made of the thickness of the film. From a consideration of the shadowing angle, the width of the dark areas, and the magnification, the thickness of the oxide is estimated to be about 100 Angstrom units.

The film grows on the iron during the electropolishing and drying process,

since the specimen of Fig. 2, which was covered with plastic as soon as it was dry, still appears to have a film. From the data in this study it is not possible to determine whether the oxide is formed during the polishing process or during the drying. Such a decision would be of value to the electrochemist since there is at present no adequate theory of electropolishing. The view that an oxide film arises in the polishing process would be in accord with that of some authors who believe that the presence of a film is necessary before polishing, as distinct from etching, begins (9).

A second feature of the photographs is the presence of what would appear to be grain boundaries. The boundary in Fig. 2 is very sharp and separates regions of different texture. The inability of the electron microscope to give more than a surface view makes it difficult to decide whether or not the grains in the oxide are based on those in the metal.

The oxidation of an electropolished surface lends to further oxide growth on top of the oxide formed by electropolishing. The surface retains its relatively smooth character. This is shown in photographs 6 and 7. However, the reduction of an electropolished surface forms a roughened surface as shown in photographs 3, 4, and 5 and re-oxidation of this surface leads to a relatively bumpy oxide film. This should lead to a higher initial oxidation rate with a reduced surface than with an electropolished surface although the final oxidation rate may be the same in both cases. This large increase in initial oxidation rate on a reduced surface has been shown to exist by microbalance measurements which will be published at a later date. The effect of the film due to electropolishing on the oxidation rate will depend on the severity of the oxidizing conditions and will be most marked when these conditions are mild. Once the thickness of oxide has been increased to the point at which further growth takes place by a diffusional mechanism, the effect of the original surface condition will be minimized. The effect of surface area will also be most apparent in the early stages of oxidation.

It is again emphasized that the results found here are connected with one type of iron and with one formula of electropolish bath. Yet the view is held that film formation nearly always accompanies electropolishing and that in those cases where its absence has been noted, the fault lies with the means of detection.

ACKNOWLEDGMENT

The authors wish to express their indebtedness to Dr. Boswell of the Mines Branch, Department of Mines and Technical Surveys, Ottawa, for his assistance and advice in developing a method of making replicas. Thanks are due to Mr. R. I. Spearman for his aid in the experimental work of this study. The electron micrographs were made by Dr. W. Barnes and Mr. W. Forsythe of the Physics Division, National Research Council of Canada, and we wish to thank them for their willing cooperation and careful examination of specimens.

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THE INDEPENDENT YIELD OF Xe^{135} PRODUCED IN THE FISSION OF NATURAL URANIUM BY PILE NEUTRONS[1]

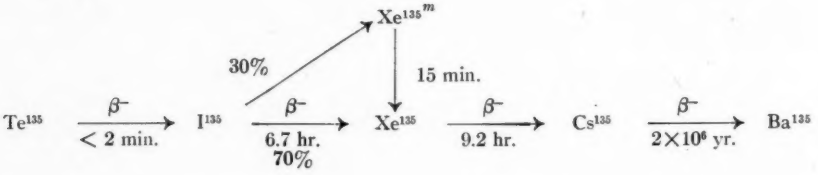
By F. Brown and L. Yaffe[2]

ABSTRACT

Fission of uranium yields Xe^{135} by two routes: direct production and production by the β-decay of I^{135}. The amount produced directly (independent yield) has been measured by comparison with the amount produced from I^{135}. The independent fission yield of Xe^{135} is found to be $2.7 \pm 1.0\%$ of the cumulative yield of I^{135}. Assuming this latter quantity to be 5.6% of all fissions, the independent yield of Xe^{135} is $\backsim 0.15\%$. The half-life of Xe^{135} has been measured as $9.13 \pm .05$ hr.

INTRODUCTION

The fission product chain with which these experiments are concerned is the following:



The independent yield of Xe^{135} has been measured by Hoagland and Sugarman (5) who found it to be about 5% of the mass 135 chain. The cumulative fission yield of the chain up to and including I^{135} was taken as 5.6% (4, 7) so that the primary yield of Xe^{135} was $\sim 0.3\%$. Adding this to the cumulative yield of I^{135}, one obtains the cumulative yield of the mass 135 chain up to and including Xe as 5.9% of fissions. The value for the independent yield of Xe^{135} obtained by Hoagland and Sugarman would not be significantly changed by making a correction for the removal of Xe^{135} by neutron capture during irradiation since the time of irradiation was short (two minutes).

The cumulative yield of the mass 135 chain up to and including Cs^{135} has been measured by Thode (9) and found to be 2.15 times that of Xe^{131}. Corrections were made for the loss of Xe^{135} by neutron capture during the irradiation.

Recent work in these laboratories (1) has given a value of 3.1% for the cumulative fission yield of I^{131}. Assuming therefore that the cumulative yield of Xe^{131} is 3.1%, we obtain the cumulative yield of Cs^{135} as 6.67%. The value of 3.1% for the cumulative yield of I^{131} is based upon the yield of Ba^{140} taken as 6.1%. The value of 5.6% for the cumulative yield of I^{135} (4, 7) is also based upon a value of 6.1% for Ba^{140}.

Thus we have the data for the mass 135 chain reduced to one single comparison substance (Ba^{140}) and the following values result: cumulative yield of I^{135} — 5.6%, cumulative yield of Xe^{135} — 5.9%, cumulative yield of Cs^{135} — 6.67%. If

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correct, these figures demand that the primary fission yield of Cs^{135} be very much higher than that allowed by any reasonable charge distribution in fission theory (3). An investigation has been undertaken of both the cumulative yield of I^{135} and the primary yield of Xe^{135}. Since the fission yield of Ba^{140} is the standard for all these determinations, this quantity has also been redetermined (2).

EXPERIMENTAL

The principle of the experimental method is as follows. A sample of uranium is irradiated for a short time with reactor neutrons. The Xe^{135} is extracted as soon as possible after the end of the irradiation and its activity measured.This xenon consists of that formed directly in fission (independent) and some formed by the radioactive decay of I^{135}. After an interval of several hours a second extraction of xenon is made and counted. This xenon was formed only by iodine decay. From the ratio of the two amounts extracted, knowing the necessary times, half-lives, etc., one can calculate the ratio of the primary yield of Xe^{135} to the cumulative yield of I^{135}.

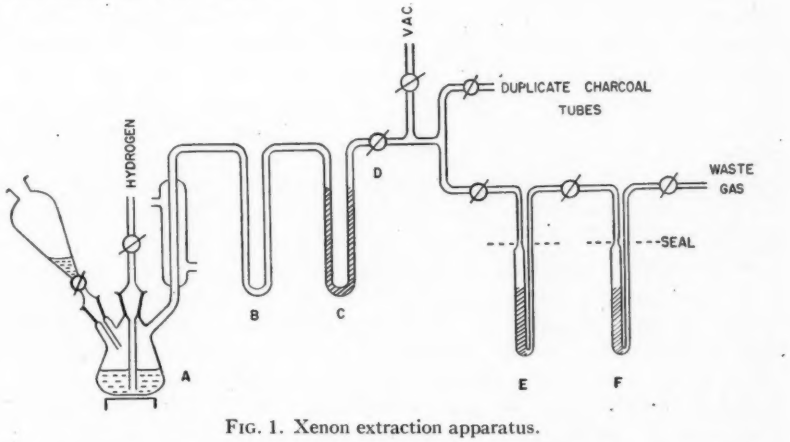

FIG. 1. Xenon extraction apparatus.

The apparatus used to dissolve the uranium or uranium compound and to collect the xenon is shown in Fig. 1. The final technique chosen for irradiation was to seal uranium oxide or uranyl nitrate in a small very thin-walled aluminum capsule. This capsule was irradiated in a high flux position (5×10^{13} neutrons/cm.^{2}/sec.) in the Chalk River N.R.X. reactor for five minutes and returned immediately to the laboratory by pneumatic conveyor. The aluminum capsule was dropped into the dissolving flask *A*, which contained about 50 mgm. of potassium iodide as "hold back carrier", and the whole apparatus was evacuated. (The charcoal traps *E* and *F* were thoroughly pumped out prior to the experiment.) Hydrogen was then admitted, followed by hot 1:1 sulphuric acid into the flask *A*. The acid rapidly dissolved the capsule and contents. The uranium solution was boiled gently while a stream of hydrogen was passed through. The effluent gas was dried by a reflux water condenser and by a trap *B* cooled in

solid carbon dioxide, after which it passed over solid sodium hydroxide in the trap *C* and thus into the collecting tubes *E* and *F*. These tubes contained activated charcoal and were cooled in a mixture of solid carbon dioxide and acetone. They were so arranged that *F* acted as guard tube for *E*. Hydrogen was passed through until the time elapsed from the end of irradiation was 30 min., the stopcock *D* was then closed, and the hydrogen and heat turned off. The tubes *E* and *F* were evacuated to a pressure of less than 5 microns mercury and then, with coolant not yet removed, they were sealed off on both sides by a flame as shown in Fig. 1. The sealed tubes containing the first gas sweep were now ready for counting as described below. After an interval of about three and one-half hours the hydrogen sweep was restarted, the solution boiled, and the gases swept into the duplicate charcoal tubes. When the time elapsed since the end of irradiation was just four and one-half hours, the stopcock *D* was again closed, and the tubes were evacuated and sealed as before.

The Xe^{135} was counted in a Shonka type ionization chamber (8). In this instrument the sample is placed inside a tube so that it is at the center of the sensitive volume. Thus the counting rate is not sensitive to small changes in the position of the sample. This was checked experimentally and no detectable change in counting rate resulted when the sample was moved through the maximum amount permitted by the counter construction. The output from the ionization chamber was amplified by a vibrating reed instrument (Applied Physics Corporation, Model 30) and this output fed on to a Speedomax recorder. Shunts were provided which could be varied so as to make the instrument cover a wide range of disintegration rates. The walls of the central tube containing the sample were of iron, approximately $\frac{1}{4}$ in. thick, so that the instrument counted almost entirely γ-rays from the sources. Background counts were taken at frequent intervals during the counting and the necessary corrections made.

The gases swept from irradiated uranium by the method employed consist of various krypton and xenon isotopes. Under the circumstances of the irradiation and counting technique used, the decay curve of the gases shows three components; a short lived one of three to four hours associated with Kr^{85} and perhaps traces of other nuclides, the Xe^{135} component with half-life about nine hours (see below for discussion of this half-life), and a long-lived component of 5.3 days half-life due to Xe^{133}. A typical example of the decay curves obtained is shown in Fig. 2. The curves obtained for each sweep of gas were analyzed in the usual manner and the Xe^{135} activity extrapolated back to the time at which that particular sweep ended. The average value for the half-life of Xe^{135} in all experiments was 9.13 hr. (see also under Results), and this value was used to extrapolate in the final analysis of results.

With regard to the gas sweeping technique the following points were established. The activity in the charcoal tubes rises very rapidly when the sweep is commenced and then reaches an almost constant value. This indicates that the sweeping technique is rapid and efficient. The slow rise in activity which continues after the bulk of xenon has been swept through is due to continuing production of xenon from iodine. The guard tubes were found to contain short-lived activity, probably Kr^{85}, but no trace of either Xe^{135} or Xe^{133},

thus showing complete absorption of xenon in the first charcoal tube.

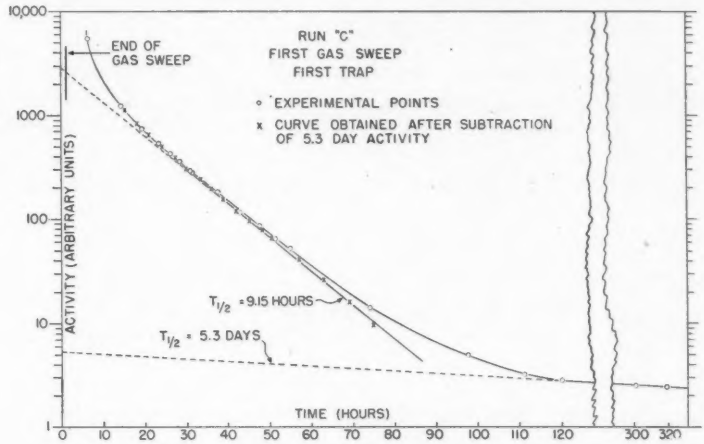

FIG. 2. Curve showing decay of first gas sample.

RESULTS

The experimental value measured is the ratio R of the Xe^{135} activity in the first gas sweep to that in the second sweep, both measured at the times of completing their respective sweeps. The first sweep contains Xe^{135} produced by decay of I^{135} and also that produced directly in fission. The second sweep contains only Xe^{135} produced from I^{135}. From this ratio R one can calculate the ratio of the primary fission yield of Xe^{135} to the cumulative fission yield of I^{135}. The method of calculation is shown below.

Let t_0 = time of the irradiation
t_1 = time elapsed between the end of the irradiation and the end of the first gas sweep
t_2 = time elapsed between the end of the first gas sweep and the end of the second gas sweep
λ_1 = decay constant for I^{135}
λ_2 = decay constant for Xe^{135}
Y = cumulative fission yield of I^{135}
Y_p = primary fission yield of Xe^{135}
N = number of fissions occurring per unit time
I_0 = amount of I^{135} present at the end of irradiation
Xe_0 = amount of Xe^{135} present at the end of irradiation
I_1 = amount of I^{135} present at the end of the first gas sweep
Xe_1 = amount of Xe^{135} present in the first gas sweep
Xe_2 = amount of Xe^{135} present in the second gas sweep.

During Irradiation

Note that during irradiation the rate of disappearance of Xe^{135} is greater than expected from its decay constant, λ_2, because a considerable quantity is con-

sumed by the reaction $Xe^{135}(n, \gamma)Xe^{136}$. The *effective* decay constant, λ_2^*, is given by

$$\lambda_2^* = \lambda_2 + \sigma\rho v$$

where σ is the neutron capture cross section of Xe^{135} and ρv is the neutron flux.

The resulting correction to the yield for a five minute irradiation is small even for the very high neutron capture cross section of Xe^{135}. It is quite negligible in the case of I^{135}.

If I is the amount of I^{135} present at any time, t, the net rate of production of I^{135} is

$$\frac{dI}{dt} = YN - \lambda_1 I$$

and the amount present after a time of irradiation t_0 is

$$I_0 = \frac{YN}{\lambda_1}(1 - e^{-\lambda_1 t_0}). \qquad [1]$$

If Xe is the amount of Xe^{135} present at any time, t, the net rate of production of Xe^{135} *from* I^{135} is

$$\frac{dXe}{dt} = YN(1 - e^{-\lambda_1 t}) - \lambda_2^* Xe.$$

The net rate of production by direct fission is

$$\frac{dXe}{dt} = Y_p N - \lambda_2^* Xe.$$

The total net rate of production during irradiation is

$$\frac{dXe}{dt} = YN(1 - e^{-\lambda_1 t}) - \lambda_2^* Xe + Y_p N$$

and the amount present at the end of irradiation t_0 is

$$Xe_0 = \frac{N(Y + Y_p)}{\lambda_2^*}(1 - e^{-\lambda_2^* t_0}) - \frac{YN(e^{-\lambda_1 t_0} - e^{-\lambda_2^* t_0})}{\lambda_2^* - \lambda_1}. \qquad [2]$$

During the Interval between the End of Irradiation and the End of the First Gas Sweep

The amount of Xe^{135} growing from an initial amount of iodine, I_0, in time t_1 is

$$\frac{\lambda_1 I_0}{\lambda_2 - \lambda_1}(e^{-\lambda_1 t_1} - e^{-\lambda_2 t_1}).$$

The amount of Xe^{135} remaining from an original amount of Xe^{135}, Xe_0, after time t, is

$$Xe_0 e^{-\lambda_2 t_1}.$$

So that the amount of Xe^{135} present at the end of the first gas sweep is

$$Xe_1 = \frac{\lambda_1 I_0}{\lambda_2 - \lambda_1}(e^{-\lambda_1 t_1} - e^{-\lambda_2 t_1}) + Xe_0 e^{-\lambda_2 t_1}.$$

Substituting for I_0 and Xe_0 from [1] and [2] we have

$$Xe = Y\left\{\frac{N}{\lambda_2 - \lambda_1}(1 - e^{-\lambda_1 t_0})(e^{-\lambda_1 t_1} - e^{-\lambda_2 t_1}) - \frac{N}{\lambda_2^* - \lambda_1}(e^{-\lambda_1 t_0} - e^{-\lambda_2^* t_0})e^{-\lambda_2 t_1}\right\}$$
$$+ (Y + Y_p)\left\{\frac{N}{\lambda_2^*}(1 - e^{-\lambda_2^* t_0})(e^{-\lambda_2 t_1})\right\}.$$

During the Interval between the End of the First Gas Sweep and the End of the Second Gas Sweep

The amount of iodine remaining at the end of the first gas sweep (beginning of second) is

[3] $$I_1 = I_0 e^{-\lambda_1 t_1}.$$

The amount of xenon present at this time is nil. Considering therefore the growth of Xe^{135} from the I^{135}, I_1, during t_2, we have the amount of Xe^{135} at the end of the second sweep given by

$$Xe_2 = \frac{\lambda_1 I_1}{\lambda_2 - \lambda_1}(e^{-\lambda_1 t_2} - e^{-\lambda_2 t_2})$$

and substituting for I_1 as in [3] and for I_0 as in [1] we have

$$Xe_2 = \frac{YN}{\lambda_2 - \lambda_1}(e^{-\lambda_1 t_1} - e^{-\lambda_1 (t_0 + t_1)})(e^{-\lambda_1 t_2} - e^{-\lambda_2 t_2}).$$

Therefore if R is the ratio of Xe^{135} in the first gas sweep to that in the second, we have

$$R = \frac{Xe_1}{Xe_2} \text{ and thus}$$

$$R = \frac{Y\left\{\frac{(1 - e^{-\lambda_1 t_0})(e^{-\lambda_1 t_1} - e^{-\lambda_2 t_1})}{\lambda_2 - \lambda_1} - \frac{(e^{-\lambda_1 t_0} - e^{-\lambda_2^* t_0})e^{-\lambda_2 t_1}}{\lambda_2^* - \lambda_1}\right\}}{Y\left\{\frac{(e^{-\lambda_1 t_1} - e^{-\lambda_1 (t_0 + t_1)})(e^{-\lambda_1 t_2} - e^{-\lambda_2 t_2})}{\lambda_2 - \lambda_1}\right\}}$$
$$+ \frac{(Y + Y_p)\left\{\frac{(1 - e^{-\lambda_2^* t_0})e^{-\lambda_2 t_1}}{\lambda_2^*}\right\}}{Y\left\{\frac{(e^{-\lambda_1 t_1} - e^{-\lambda_1 (t_0 + t_1)})(e^{-\lambda_1 t_2} - e^{-\lambda_2 t_2})}{\lambda_2 - \lambda_1}\right\}}$$

$$R = 0.2063 + 3.385\,\frac{Y_p}{Y}$$

if one uses the following values:

$t_0 =$ 5 min. half-life $I^{135} = 6.7$ hr.
$t_1 =$ 30 min. half-life $Xe^{135} = 9.15$ hr.
$t_2 =$ 240 min. σ for $Xe^{135} = 3.5 \times 10^{-18}$ cm.^{2}
$\rho v = 5 \times 10^{13}$ neutron/cm.^{2}/sec.

Four runs were made using the standard procedure outlined above.
Run A employed 260 mgm. of uranium metal in three pieces, not sealed.

Run B employed 260 mgm. of uranium oxide, UO_3, sealed in a small aluminum capsule.

Run C employed 250 mgm. of uranium oxide as in Run *B*.

Run D employed 250 mgm. of uranyl nitrate, $UO_2(NO_3)_2.6H_2O$, also sealed in an aluminum capsule.

The following Table I gives the measured values of R, the ratio of activities as defined above and also the values of Y_p/Y calculated from them, where Y_p is the primary fission yield of Xe^{135} and Y is the cumulative fission yield of I^{135}.

TABLE I

Run	R	$\frac{Y_p}{Y} \times 100$	$t_{1/2}$ Xe^{135} (hr.)
A	0.251	1.33	9.12 9.11
B	0.366	4.72	9.18 9.06
C	0.234	0.82	9.15 9.09
D	0.340	2.70	9.23 9.10
Average	0.298	2.7	9.13

The average value for R is 0.298 with a standard deviation of 0.033. The corresponding value for Y_p/Y is $2.7 \pm 1.0\%$. Assuming $Y = 5.6\%$ of fissions (4, 7) the primary fission yield of Xe^{135} would be $0.15 \pm 0.06\%$ of fissions. It is important to note, however, that the value for Y_p/Y is very sensitive to small changes in the experimental quantity R when Y_p is very small compared to Y. In fact, the primary yield of Xe^{135} is too small to be measured satisfactorily by the method used. One is essentially measuring a small difference between two large quantities which can not themselves be measured with high accuracy. The results therefore should be taken to indicate that the primary fission yield of Xe^{135} is very small but finite.

Each of the four runs yielded two values for the half-life of Xe^{135} making eight values in all (Table I). The average value is 9.13 hr. with a standard deviation of $\pm$ 0.02 hr. The value given by Hoagland and Sugarman (6) is 9.2 hr., being based upon measurements with a Geiger tube which gave 9.3 hr. and measurements with an ion chamber which gave $9.15 \pm .05$ hr. A value of 9.1 hr. was obtained by Thulin, Bergström, and Hedgran (10) using a mass spectrometer. All these results are in satisfactory agreement and one should perhaps assume a value of $9.15 \pm .05$ hr.

DISCUSSION

This result is in general agreement with that of Hoagland and Sugarman (5). The independent yield of Xe^{135} is too small to account for the difference in the cumulative yields of I^{135} and Cs^{135} as measured (4, 7, 9). The reason for this discrepancy has yet to be found.

The independent yield of Xe^{135} is one of the points used by Glendenin, Coryell,

and Edwards (3) to fix the position of the curve showing the variation of fission yield with nuclear charge. Since this is plotted on a logarithmic scale, the use of our value, which is lower than the Hoagland and Sugarman one, will not have much effect. It is in the direction of giving even better agreement with the equal charge displacement theory than before.

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BETATRON IRRADIATION OF AQUEOUS FERROUS SULPHATE SOLUTIONS[1]

By R. W. Hummel and J. W. T. Spinks

ABSTRACT

The oxidation of aerated aqueous solutions of ferrous ammonium sulphate has been studied using radium gamma rays and X rays of 24.5 Mev. peak energy from a betatron. The behavior at high photon energies has been shown to be closely similar to that at lower photon energies.

INTRODUCTION

In recent years the study of radiation chemistry has acquired a new importance. The effects of new radiations have been investigated and in some cases the range of older observations has been extended. In the present paper, the oxidation of ferrous sulphate solutions by the X rays from a betatron is reported.

DOSIMETRY

The dose unit universally adopted is the roentgen which is officially defined as "that quantity of X- or gamma radiation such that the associated corpuscular emission for 0.001293 gram of air produces, in air, ions carrying one electrostatic unit of quantity of electricity of either sign." The mass of air referred to is 1 cc. of dry air at 0°C. and 760 mm. Hg.

Special techniques have been developed in order that radiation dosages may be measured in compliance with the requirements of the definition. In this work, the Victoreen thimble chamber was used for dosimetry.

The original idea underlying the thimble chamber method was supplied by Bragg (3), but the first experimental application was apparently that of Fricke and Glasser (6). Gray (9, 10) is generally credited with developing the theory on which the air-wall chamber is based and has suggested (11) that accuracies of better than 5% are attainable. A recent discussion of the theory and of the factors affecting accuracy has been given by Wang (24).

With high energy photons from the betatron a very thick-walled chamber is necessary in order to satisfy the equilibrium conditions demanded by the Bragg–Gray relation. For instance, the depth–dose curve for water indicates that for 25 Mev. X rays the dose is greatest at a depth of about 4 cm. (17). In contrast, when using a gamma ray source and an air-filled chamber, a wall thickness of about 4 mm. of graphite is sufficient (19). The effective atomic number of Lucite is not very different from that of water, and consequently a thickness of 4 cm. of Lucite has been used in dosage determinations with betatron X rays (4). A roentgen measured with a Lucite wall chamber is sometimes called a "Lucite roentgen."

An expression for the value of a roentgen in terms of a "Lucite roentgen" can be derived from the Bragg–Gray relationship, $E_v = J_v W \rho$, where E_v = energy

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Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Sask.

absorbed per cm.^{3} by the medium, J_v = number of ionizations per unit volume of air, W = energy required to form an ion pair, taken as 32.5 ev. (18), and ρ = ratio of the stopping power of the medium to that of air. It is found that:

$$\frac{\text{energy giving a roentgen}}{\text{energy giving a Lucite roentgen}} = \frac{(_e\tau_a + {}_e\sigma_a + {}_e\pi_a)_{\text{Lucite}}}{(_e\tau_a + {}_e\sigma_a + {}_e\pi_a)_{\text{air}}} \cdot \frac{{}_eS_{\text{air}}}{{}_eS_{\text{Lucite}}}$$

where ${}_eS_{\text{air}}/{}_eS_{\text{Lucite}}$ = ratio of electronic stopping powers of air and Lucite and ${}_e\tau_a$, ${}_e\sigma_a$, and ${}_e\pi_a$ are the real electronic absorption coefficients for the photoelectric, Compton, and pair production processes, respectively.

The "Lucite roentgen" is measured by having the Victoreen thimble chamber in a Lucite block. The ratio of the "Lucite roentgen" to the true roentgen has been calculated for the energy range, 0.1 to 25.5 Mev., by the betatron group at this University. The variation with energy of this ratio makes it necessary to calculate an average value weighted according to the relative intensity of the X rays at different energy levels. The spectral distribution of photon energies of the X rays coming from the betatron, at a maximum energy of 24.5 Mev., is shown in Fig. 2, and the relative intensities at several energy levels are estimated from curve *B* of this figure. The values obtained are given in Table I.

TABLE I

Energy level (Mev.)	Relative intensity
24	6.3
20	16.9
16	21.9
12	25.0
8	28.8
4	31.9
2	32.2
1	30.6

TABLE II

RELATIONSHIP BETWEEN A "LUCITE ROENTGEN" AND A TRUE ROENTGEN FOR 24.5 MEV. PEAK ENERGY BETATRON RAYS

(1) Energy region, Mev.	(2) Fraction of bremsstrahlung in each region	(3) Energy giving a roentgen / Energy giving a "Lucite roentgen"	(4) (2) × (3)
0.1- 1	0.051	0.95	0.0485
1 - 2	0.103	0.979	0.101
2 - 4	0.106	0.972	0.103
4 - 6	0.103	0.956	0.0985
6 - 8	0.098	0.938	0.0919
8 -10	0.092	0.923	0.0849
10 -12	0.085	0.912	0.0775
12 -14	0.080	0.904	0.0723
14 -16	0.075	0.896	0.0672
16 -18	0.068	0.888	0.0604
18 -20	0.060	0.881	0.0529
20 -22	0.047	0.875	0.0411
22 -24	0.031	0.870	0.0270

$\sum$ (2) × (3) = 0.926 1/0.926 = 1.08 true roentgens/"Lucite roentgen"

From a plot of these points, the average intensity of the bremsstrahlung in energy regions 2 Mev. or less in width was estimated. By dividing the values thus obtained by their sum, the fraction in each region was obtained. The sum of the products of these values and the values giving a "Lucite roentgen" in the same energy regions gave an average value for the ratio weighted in the desired manner. The results of the calculations are given in Table II.

Dosage measurements made with the Victoreen thimble chamber in the Lucite block at 24.5 Mev. peak energy were therefore increased by 8% in order to obtain the dose received in air roentgens.

EQUIPMENT

The Betatron

Fig. 1 shows the general arrangement existing during experiments with the

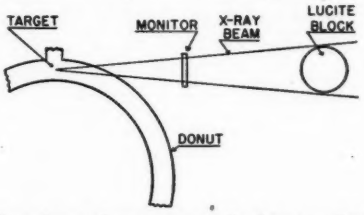

FIG. 1. Experimental arrangement for betatron irradiations.

betatron. Electrons accelerated in the donut are allowed to strike a tungsten target. The *bremsstrahlung* produced by deceleration of the electrons has a continuous spectrum of energies ranging from 25 Mev., the maximum kinetic energy of the electrons—less 0.5 Mev. rest energy—to zero energy. The shape of the spectrum at a given maximum energy may be calculated from a formula due to Schiff (22).

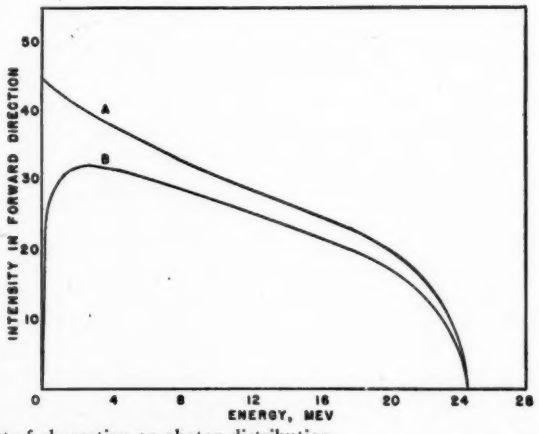

FIG. 2. Effect of absorption on photon distribution.
A. Theoretical Schiff distribution for the maximum photon energy of 24.5 Mev.
B. Curve *A* modified by absorption of donut, monitor, and Lucite block using real absorption coefficients.

The spectral distribution as obtained is modified by absorption due to the walls of the donut, the monitor, and the Lucite block used to hold samples being irradiated. This is illustrated in Fig. 2, where curve *A* is the theoretical Schiff distribution for a maximum photon energy of 24.5 Mev. and curve *B* is curve *A* modified by absorption due to donut, monitor, and Lucite block, and is a fairly close approximation to the distribution to which the Victoreen responds. Curve *B* was calculated for an 8 cm. cube of Lucite with a 1.8 cm. hole drilled to a depth of 5 cm. in the center of one face. The circular Lucite block in which the ferrous iron solutions were irradiated in this study is described later and will, of course, give a slightly different distribution.

On operating the betatron an ionization current is induced in the monitor through which the photon beam passes. The current charges a condenser which operates a counter and relay when its potential reaches a predetermined value. The relay operates a shorting circuit which discharges the condenser. Each count or "click", therefore, represents a definite amount of radiation which has passed through the monitor. The number of "Lucite roentgens" per click can be determined by placing a Victoreen r-meter in the sample position and taking the average of several measurements. The dose rate is obtained by timing the click rate with a stop watch.

Apparatus for Betatron X-Ray Irradiations

Irradiations with betatron X rays were carried out in 13 × 100 mm. Pyrex test tubes inserted in the Lucite block illustrated in Fig. 3. The block was almost

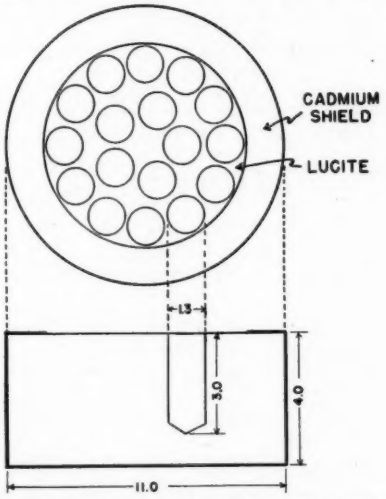

FIG. 3. Apparatus for irradiations with the betatron. Dimensions in centimeters.

completely covered with sheet cadmium* about 0.4 mm. thick, a space being left uncovered at the top to allow insertion of the sample tubes. A wooden pulley wheel was fastened to the bottom of the block, a smaller pulley wheel was

**The cadmium was intended to stop the slow neutrons present in both γ-ray and betatron X-ray beams.*

mounted on a synchronous motor whose rate of rotation was 240 r.p.m., and the two were connected by a cord. The pulley wheels were made so that the Lucite block would rotate at about 30 r.p.m. The mountings for the Lucite block and the synchronous motor were clamped to an ordinary laboratory stand. The stand was placed on a platform fastened to the betatron, and the block adjusted as closely as possible to the center of the aperture from which the X rays emerged. A clamp held the stand rigidly to the platform during an irradiation so that the position of the block relative to the beam would not be altered. This method of positioning the block was not completely reproducible, but, since a dose determination was carried out at the beginning of each run, no error resulted thereby.

A rotating block for the betatron experiments was chosen because the X-ray intensity was not constant across the beam, and hence a stationary block would receive different dosages at different points. In addition, the direction of the beam has been known to shift slightly during operation of the betatron, whence the dose rate at any point in a stationary block would change as often as the beam changed direction. With the rotating system, variations in intensity across the beam, or changes in beam direction, or attenuation due to varying thicknesses

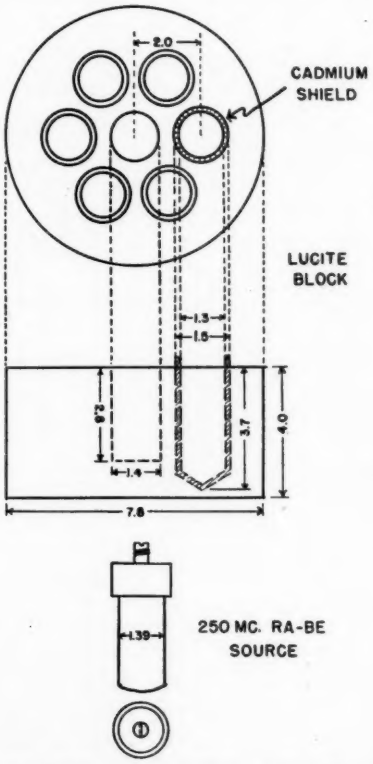

FIG. 4. Apparatus for irradiations with gamma-ray source. Dimensions in centimeters.

of Lucite traversed by the beam, did not have to be allowed for and each sample in a given circle received the same dose.

Gamma-ray Source

The gamma-ray source available to us was a 250 mc. Ra–Be mixture. This source was also a neutron emitter. The possible effect of these neutrons was investigated by carrying out several experiments in which some of the sample tubes were placed in cadmium shields and others in Lucite shields of identical geometry. The results showed no significant differences, and it was assumed that oxidation due to slow neutron bombardment was comparatively unimportant. However, cadmium shields were used in all subsequent experiments as a precautionary measure.

Apparatus for Gamma-ray Irradiations

Irradiations with the 250 mc. gamma-ray source were carried out in 13 × 100 mm. Pyrex test tubes inserted in the Lucite block illustrated in Fig. 4. The gamma-ray source was inserted in the central hole and the tubes placed in the cadmium shields in the six surrounding positions.

ANALYTICAL METHOD

Ferrous ion concentrations were determined colorimetrically using orthophenanthroline (2). To develop the color, orthophenanthroline solution was added to the iron solution, and finally enough sodium citrate solution was added to bring the pH to about 4. The pale yellow ferric citrate complex did not interfere with the analyses since the iron solutions were not irradiated enough to oxidize more than three-quarters of the ferrous ions. Sodium acetate buffer was found to be unsatisfactory in this respect (15).

A calibration curve was established for ferrous–orthophenanthroline systems buffered with sodium citrate at about pH 4. The extinction coefficient at 510 mμ was 11,550 ± 30, using a Beckman model D.U. spectrophotometer.

Laboratory distilled water was redistilled first from alkaline permanganate solution and then from alkaline manganous hydroxide suspension. Pyrex glass was used throughout.

The analytical procedure was carried out as follows. To aliquots of the iron solution were pipetted first a slight excess of 0.1% orthophenanthroline solution and then sufficient sodium citrate solution to buffer at a pH of about 4. Dilution in a volumetric flask was effected when necessary. The importance of adding the indicator before the buffer has been pointed out by Bandemer and Schaible (1). The optical density was determined at 510 mμ, and the concentration found from the calibration curve.

For an irradiation, 3- or 2-ml. aliquots, depending on whether radium or the betatron, respectively, was used as the radiation source, were transferred with a calibrated pipette to 13 × 100 mm. Pyrex test tubes which had been thoroughly cleaned with hot chromic acid solution and rinsed at least three times each with tap water, distilled water, and triple distilled water and then air-dried.

After irradiation, the ferrous ion concentration was determined in the same manner as before.

EXPERIMENTAL RESULTS

Dosage Determinations

Victoreen condenser thimble chambers were used to determine dosages in roentgens. With the gamma-ray source a 25 r. chamber was used, while with the betatron a 100 r. chamber was found necessary.

To determine dosages obtained from the gamma-ray source, the source was placed in the center hole of the Lucite block shown in Fig. 4. The 25 r. thimble chamber was placed in turn in each of the six surrounding holes, and the dose received in three minutes read off the scale on the Victoreen charging unit. It was possible to take two sets of readings in the above manner, the first set with the sensitive volume of the chamber in the upper half of the hole, and the second set with the sensitive portion in the lower half. The average of these two sets of dose determinations was taken as the average dose received throughout the hole, and consequently, since the 3-ml. portions of ferrous sulphate solution used in this study almost completely filled the space in which the dose determinations were made, as the dose received by the irradiated solutions.

The Victoreen reading was corrected for temperature and pressure, for the change in calibration of the chamber as determined independently using a set of radium needles, and for the fact that the measurements were made in a Lucite block. The latter correction factor was + 2% and was estimated from Table II by assuming that the important gamma rays from radium have energies between 0.3 and 2 Mev. The corrected dose rate was 430 r. per hr.

To determine dosages with the betatron source of X rays, the 100 r. chamber was used. By experiment, this chamber gave 3% higher values at 23 Mev. than did the 25 r. chamber. The dosages obtained with the 100 r. chamber were therefore reduced by 3% so that all the results reported in this work could be based on dosages determined with the 25 r. chamber. Dosages were determined at the start of an experiment by inserting an empty test tube into one of the positions in the Lucite block (Fig. 3), the tube being shortened considerably in order to accommodate the thimble chamber. Tubes containing 2 ml. of water were placed in the other positions. The block was rotated at about 30 r.p.m., five or more clicks at a known maximum X-ray energy were given, and the dose read off the Victoreen unit. Several measurements were made at any one position in both the inner and outer circles of holes, and it was assumed that the dose received at each of the positions in a circle was the same. The average value of the dose per click for each circle was corrected for temperature and pressure, decreased by 3% to obtain the value which would have been recorded by the 25 r. chamber, and increased by 8% to convert from "Lucite roentgens" to true roentgens.

Irradiation of Ferrous Ion Solutions with Radium Gamma Rays

A series of runs at different initial ferrous ammonium sulphate concentrations, all in 0.8 *N* sulphuric acid, confirmed the results obtained by other workers in this field. A typical result is shown in Fig. 5. The over-all reaction obeyed zero order rate equations throughout the concentration range studied. The oxidation rate was independent of concentration at initial Fe^{++} concentrations greater than 5×10^{-5} molar, but decreased at lower initial concentrations. The variation of oxidation rate with initial Fe^{++} concentration is shown in Fig. 6.

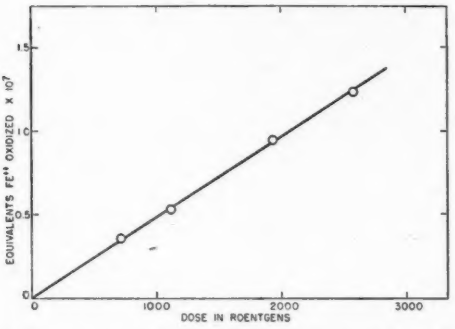

FIG. 5. Oxidation yield as a function of roentgen dose; typical result obtained with the gamma-ray source. Initial ferrous concentration = 5.48×10^{-5} *M*.

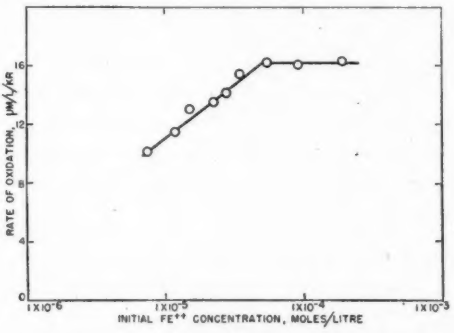

FIG. 6. Variation of oxidation rate with initial ferrous ion concentration using radium gamma rays.

The rates are expressed in terms of micromoles ferrous ion oxidized per liter of solution per 1000 roentgens. The oxidation yields are also often expressed in terms of the number of ions oxidized per 100 electron volts absorbed in the solution and are represented by the letter *G*. The average value of *G* in the concentration independent region = 16.8 ions oxidized per 100 ev. at 23°C. The temperature coefficient is very low in the concentration independent region but increases when the initial concentration is less than 5×10^{-5} molar. Some results at low concentrations are given in Table III. The change in the oxidation rate at 25°C. was about 0.2 μM./l./kr./°C. for the 1.32×10^{-5} *M* solution. Minder and Liechti (21) did not find a variation in the rate from 4°C. to 54°C., owing to their use of a 2 *M* solution. The average rate in the concentration independent region is compared with the rates obtained using the betatron and with values obtained by other workers, in Table V (see below).

Irradiation of Ferrous Ion Solutions with Betatron X Rays

The dependence of oxidation rate on initial ferrous ion concentration was investigated in the same manner with the betatron as with the radium source. Two-milliliter aliquots of each solution were irradiated.

TABLE III

EFFECT OF TEMPERATURE ON OXIDATION RATE

Initial Fe^{++} concentration, M	Temperature, °C.	Oxidation rate, $\mu M./l./kr.$
1.32×10^{-5}	84	16.2
"	61	16.2
"	27	12.9
"	12.5	9.0
1.19×10^{-5}	2	8.1

After determining the number of roentgens per click, the ferrous ion solutions, in Pyrex test tubes, were placed in the Lucite block, the block was rotated, and a known number of clicks given to each sample tube. As each tube was removed, a similar tube containing 2 ml. of water was put in its place. Since the number of clicks for each sample as well as the number of roentgens per click were known, the dosage was readily calculated. The dose per click at the end of the run was checked on several occasions, but no change from the initial value was observed.

At 24.5 Mev. peak energy the dose rate at the inner circle of sample holes in the Lucite block was about 400 r. per min. while at the outer circle the dose rate approximated 280 r. per min. These rates were not constant throughout a run, the number of clicks per minute being determined by manual setting of the betatron controls. At 18 Mev. peak energy the dose rate dropped to about 110 r. per minute and 120 r. per minute at the outer and inner circles, respectively.

A number of ferrous solutions of different initial concentrations were irradiated with 24.5 Mev. peak energy X rays. The results were very similar to those obtained with radium gamma rays. The results of a typical run are shown in Fig. 7.

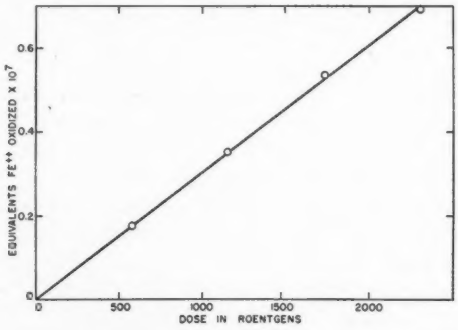

FIG. 7. Oxidation yield as a function of roentgen dose; typical result obtained with betatron X rays at 24.5 Mev. peak energy. Initial ferrous ion concentration = 5.38×10^{-5} M.

At every initial concentration a zero order rate equation was obeyed. The rates of oxidation were expressed in the usual way and are plotted in Fig. 8 against the initial ferrous ion concentrations. A comparison with Fig. 6 shows that the behavior of these solutions at 24.5 Mev. peak X-ray energy is very similar to their behavior at much lower energies. For example, in both cases the rate is constant above initial ferrous ion concentrations of about 4×10^{-5} M and decreases as

the concentration is decreased below that value. There appeared to be no difference in rate of ferrous ion oxidation between samples irradiated in the inner or outer holes.

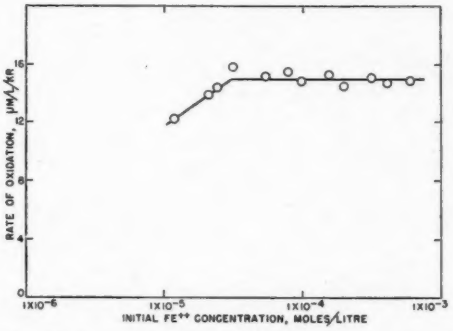

FIG. 8. Variation of oxidation rate with initial ferrous ion concentration using betatron X rays at 24.5 Mev. peak energy.

Several experiments at different initial concentrations were carried out at 18 Mev. peak X-ray energy. The rates of oxidation are essentially the same as were found with 24.5 Mev. X rays. The results of the betatron experiments are tabulated in Table IV.

TABLE IV

RATE OF OXIDATION AS A FUNCTION OF INITIAL CONCENTRATION (BETATRON X RAYS)

Initial ferrous ion concentration, *M*	Peak energy, Mev.	Oxidation rate, μM./l./kr.
6.00×10^{-4}	24.5	14.9
$4.10 \times$	24.5	14.7
$3.15 \times$	24.5	15.1
$2.00 \times$	24.5	14.5
$1.55 \times$	24.5	15.3
9.90×10^{-5}	24.5	14.9
$7.90 \times$	24.5	15.5
$5.38 \times$	24.5	15.2
$3.16 \times$	24.5	15.9
$2.44 \times$	24.5	14.4
$2.11 \times$	24.5	13.9
$1.19 \times$	24.5	12.3
2×10^{-4}	18.0	15.3
7.90×10^{-5}	18.0	15.5
$3.16 \times$	18.0	15.1

They are compared with gamma-ray results obtained in the present experiments and by other workers, in Table V.

TABLE V

COMPARISON OF G VALUES FOR DIFFERENT WORKERS

Workers	Type of radiation	Oxidation rate, G
Fricke *et al.* (7, 8)	X rays	18.0
Shishacow (23)	X rays	15.9
Miller (Polystyrene cells) (20)	Radium gamma rays	20.4
Miller (Pyrex cells) (20)	Radium gamma rays	18.2
Johnson (16)	2 Mev. X rays	17.3
Hardwick (Polystyrene cells)* (12)	Co^{60} gamma rays	20.8
Hardwick (13)	Beta rays, P^{32}	21.1
Hardwick (13)	Beta rays, S^{35}	20.2
Hochanadel and Ghormley (14)	Co^{60} gamma rays	15.7
Freeman *et al.* (5)	Co^{60} gamma rays	17.4
Present work	Radium gamma rays	16.8
Present work	Betatron X rays 24.5 Mev. peak energy	15.8

**Using essentially the same equipment as Miller.*

DISCUSSION

The primary purpose of this study was to extend the field of radiation chemistry, in particular the radiation chemistry of aqueous ferrous ammonium sulphate solutions, to high energy X-ray regions which heretofore have not been reported in the literature.

The behavior of aerated aqueous ferrous ammonium sulphate solutions made 0.8 N to sulphuric acid has been shown here to be similar at high photon energies to that found at the low photon energies associated with X-ray machines and gamma-ray sources. That is, in the concentration range above 5×10^{-5} M and for temperatures at or above room temperatures, the rate of oxidation is independent of concentration and in a given experiment the amount of ferrous ion oxidized is proportional to the energy absorbed. In addition, the yield per ion pair is nearly the same at both low and high photon energies. Hence, the mechanism whereby X rays cause oxidation of ferrous ion in aqueous sulphuric acid solutions is probably the same with the 24.5 Mev. peak X-ray beam as at low X-ray or gamma-ray photon energies (< 3 Mev.). Since the mechanism at low photon energies is thought to involve oxidizing intermediates formed by disruption of water molecules and does not result from "direct hits" by X-ray photons on the ferrous ions, then the same oxidizing intermediates may be postulated at these higher energies. The behavior of the solutions at concentrations below 5×10^{-5} M has been shown to be parallel to the behavior found at lower energies in that with both the 24.5 Mev. peak energy X-ray beam and radium gamma rays the rate of oxidation decreases with decreasing initial concentration.

The slightly lower yield obtained with betatron radiations at initial ferrous ion concentrations greater than 10^{-4} M, compared with the yield obtained with radium gamma rays, may be due to the pulsed nature of the betatron beam. The pulses are of about 4 μsec. duration and occur 180 times each second. Thus for a measured dose rate of 10^4 r. per hr. the dose rate in a pulse is about 14×10^6 r. per hr. In this connection, Miller (20) has indicated that the ferrous ion system is dose-rate dependent above 0.36×10^6 r. per hr. while Hochanadel and Ghormley

(14) found the system dose-rate independent from 18×10^3 r. per hr. to 0.81×10^6 r. per hr. with initial ferrous ion concentrations greater than 10^{-4} *M*. Thus the situation with respect to dose rate is still ambiguous and in addition the highest dose rate reported is much lower than is obtained during a betatron pulse.

ACKNOWLEDGMENT

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REACTIONS IN DISSOCIATED PEROXIDE VAPOR[1]

By J. S. Batzold,[2] C. Luner,[3] and C. A. Winkler

ABSTRACT

The products of the electrical discharge through hydrogen peroxide vapor were hydrogen peroxide, water, oxygen, and hydrogen, in amounts which depended upon the arrangement and temperature of the trap, reaction time, and surface to volume ratio of the reaction vessel. Water, hydrogen, and oxygen resulted from the gas phase reactions of the dissociated hydrogen peroxide, with hydrogen peroxide produced only in a trap cooled below − 120°C. Products trapped below − 150°C. evolved oxygen on warming to room temperature. The decomposition products of the electrical discharge through hydrogen peroxide correspond closely with products obtainable both from a similar discharge through water vapor and from the interaction of hydrogen atoms with oxygen molecules in a cold trap. A mechanism which accounts for their correspondence is included. Water was the only product when molecular hydrogen peroxide was caused to react with hydrogen atoms, dissociated hydrogen peroxide vapor, or dissociated water vapor in the presence or absence of molecular hydrogen. A chain mechanism is postulated for these reactions.

INTRODUCTION

When water vapor is dissociated in an electrical discharge, the yields of products depend upon experimental conditions such as trap temperature, reaction time, and ratio of surface to volume in the reaction chamber, in the manner outlined in a previous paper from this laboratory (5). The product trapped at liquid nitrogen temperature was found to evolve oxygen when warmed above − 120°C. In this respect, the product of the discharge through water vapor showed the same behavior as products obtained by Geib and Harteck from the reaction of hydrogen atoms with oxygen molecules in a cold trap (3). To account for the similar behavior in the two systems, a mechanism was suggested involving an unstable collision complex [HOOH]*, formed either from two OH radicals, or from two hydrogen atoms and an oxygen molecule, similar to the activated complex proposed by Badin for the low temperature hydrogen atom – oxygen molecule reaction (1).

Since production of hydroxyl radicals by an electrical discharge through water vapor (6, 7) involves the simultaneous production of hydrogen atoms, while the similar dissociation of hydrogen peroxide vapor yields only hydroxyl radicals (2), a study has been made of the reactions in dissociated hydrogen peroxide vapor to enable comparison to be made with the reactions in dissociated water vapor studied previously. The results are summarized in the present paper together with the results of some studies on reactions of hydrogen peroxide vapor with hydrogen atoms and with dissociated water and peroxide vapors.

EXPERIMENTAL

The apparatus was a conventional flow system essentially identical with that described by Jones and Winkler (5). Commercial 90% hydrogen peroxide

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[2] *Holder of National Research Council Fellowship.*
[3] *Holder of a Scholarship under the Bureau of Scientific Research of the Province of Quebec.*

(Buffalo Electro-chemical Co.)* was distilled twice *in vacuo* to obtain a product better than 99% hydrogen peroxide by weight, (analyzed by permanganate titration). A constant pressure of hydrogen peroxide vapor (about 4 mm. Hg) was obtained at the inlet side of the flow meter by maintaining a reservoir of liquid peroxide in a water bath at 34.2°C. The flow meter was heated to 35°C. in an air bath to prevent condensation of peroxide vapor.

In most of the experiments, the rate of flow into the discharge tube was 7.4 mM. of hydrogen peroxide per hour, together with 1.4 mM. of water vapor per hour originating from the unavoidable presence of small amounts of water in the liquid peroxide. The discharge potential varied from 900 to 1200 volts at 200 milliamperes, depending on the pressure in the discharge tube, which was usually close to 0.1 mm. of Hg.

RESULTS

Influence of Trap Temperature

Preliminary experiments showed that no molecular hydrogen peroxide survived the discharge, since a trap cooled in dry ice collected only water at all the flow rates used. Thus, all hydrogen peroxide collected at lower trap temperatures could be attributed to reactions in the cold trap itself. Experiments were made at intervals of 10°C. over the range of trap temperatures between − 195°C. (liquid nitrogen) and − 78°C. (dry ice – acetone). Liquid oxygen provided a trap temperature of − 183°C., while other temperatures were obtained, and regulated to within 0.2°C. of the desired temperature, by addition of small amounts of liquid nitrogen to a test tube immersed in liquid propane. After each experiment the product was warmed to 0°C. and the evolved oxygen determined manometrically. After the trap and its contents were weighed, the yield of hydrogen peroxide was determined by permanganate titration. The water yield was calculated by difference. The yields of molecular oxygen and hydrogen were also obtained by difference, from the known input of hydrogen peroxide and water. The results are shown Fig. 1, which also includes the results obtained by Jones and Winkler with a discharge through water vapor. Their experimental values have been adjusted to the production of OH radicals (16.2 mM. per hr.) in the discharge through hydrogen peroxide vapor.

The products of both systems collected at the same trap temperature evolved the same amounts of oxygen. The yields of hydrogen peroxide were essentially the same at trap temperatures between − 120°C. and − 170°C., below which temperature the peroxide yield from the water system became noticeably larger. The amount of water obtained from each system was roughly equivalent at trap temperatures between − 78°C. and − 120°C., but at lower temperatures the yield from dissociated water vapor increased rapidly. Between − 160°C. and − 195°C., the yields of water were sensibly constant from both systems, with the yield from the water system about 30% larger than that from dissociated peroxide vapor.

Influence of Reaction Chamber

In a series of experiments, the dissociated hydrogen peroxide was allowed to

**Kindly supplied by Dr. P. A. Giguère of Laval University, Quebec.*

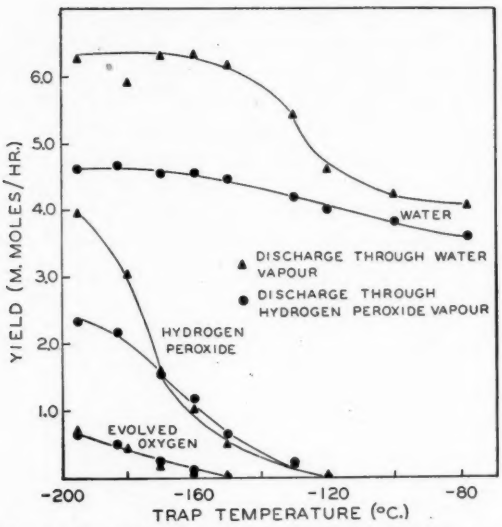

FIG. 1. Variation in the yields of product trapped with changing trap temperature for the discharge through water vapor and hydrogen peroxide vapor.

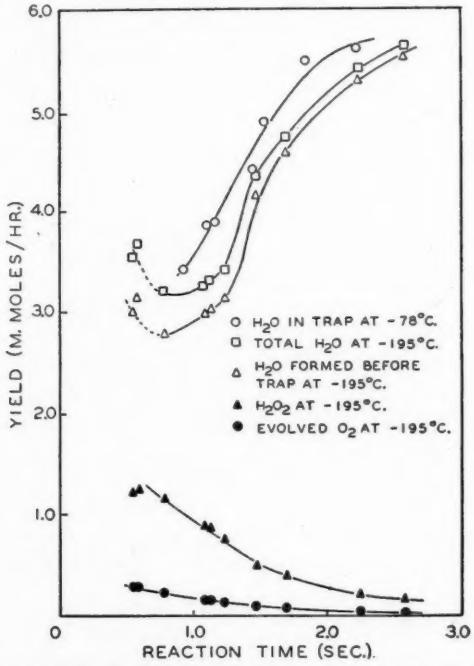

FIG. 2. Variation in yields of products with changing time in the small reaction flask for the discharge through hydrogen peroxide vapor.

react at room temperature for lengths of time which depended upon the pressure in the reaction chamber (volume = 1024 ml.) situated between the discharge tube and trap. The pressure varied linearly from 0.049 mm. for the shortest reaction time (0.57 sec.) to 0.22 mm. for the longest reaction time (2.57 sec.) with a trap temperature of − 195°C., and from 0.080 mm. to 0.182 mm. as the reaction time increased from 0.93 sec. to 2.22 sec., with a trap temperature of − 78°C.

Fig. 2 shows that the amount of water obtained in a trap cooled in dry ice was larger than the amount collected in a trap cooled in liquid nitrogen at all reaction times. The yields of hydrogen peroxide and evolved oxygen at a trap temperature of − 195°C. decreased steadily with increasing reaction time, while the yield of water increased, both at − 78°C. and − 195°C. Similar experiments with a larger reaction chamber (5400 ml.) exhibited the same behavior.

Two experiments were made at constant pressure, one with each reaction vessel, in which the surface to volume ratios of the vessels were made approximately equal (0.66 $cm.^{-1}$) by the addition of the required number of Pyrex glass rods (Table I). The results of these experiments, in which the only experimental variable was the reaction time, again showed that the yields of hydrogen peroxide and evolved oxygen were decreased, and the yield of water increased, with increased reaction time.

TABLE I

EFFECT OF REACTION TIME ON YIELDS OF PRODUCTS
TRAP TEMPERATURE: −196°C.

Volume of reaction chamber (ml.)	Reaction time (sec.)	Yields—mM. per hour				
		H_2O_2	H_2O	Evolved O_2	H_2	O_2
1024	1.21	0.43	3.78	0.07	4.59	5.71
5400	6.19	0.12	5.50	0.02	3.18	5.21

The effect of the surface area to which the dissociated hydrogen peroxide was exposed was investigated by loosely filling the small reaction chamber with 0.175 gm. of glass wool (Table II). At a trap temperature of − 195°C., the yields of hydrogen peroxide and evolved oxygen were drastically reduced, corresponding to an increase in the yield of gaseous oxygen and hydrogen. At − 78°C., the yield of water (the sole condensed product) was reduced, again with a corresponding increase in the amount of hydrogen and oxygen.

Discharge Through a Mixture of Hydrogen and Hydrogen Peroxide

The differences between the yields obtained from the hydrogen peroxide and water systems could have been caused by differences in the concentration of hydrogen atoms present in the two systems. A discharge through a mixture of hydrogen gas and hydrogen peroxide might be expected to yield products more

TABLE II

EFFECT OF SURFACE: VOLUME RATIO ON YIELDS OF PRODUCTS

	Yields: mM. per hour							
	Trap temp.: −196°C.					Trap temp.: −78°C.		
	H_2O_2	H_2O	Evolved O_2	H_2	O_2	H_2O	H_2	O_2
Unpacked vessel	0.87	3.26	0.14	4.67	5.46	3.86	4.94	6.17
Packed vessel	0.24	3.18	0.04	5.38	6.23	3.22	5.58	6.49

closely resembling those obtained with a discharge through water vapor. Hydrogen gas was passed at a steady rate through a conventional flow system and mixed with hydrogen peroxide vapor before entering the discharge tube. The input of hydrogen gas was within 5% of 16 mM. per hour in each experiment. Products were trapped at − 195°C., − 150°C., and − 78°C. in individual experiments.

In general, the results followed the expected trend (Table III). The proportions of products were closer to those obtained from the discharge through

TABLE III

YIELDS OF PRODUCTS FROM DISCHARGE THROUGH HYDROGEN PEROXIDE–HYDROGEN MIXTURES

Trap temperature (°C.)	H_2 input (mM./hr.)	Yields: mM. per hour				
		H_2O_2	H_2O	Evolved O_2	H_2	O_2
− 195°	16.30	3.70	7.02	0.56	15.05	0.33
− 150	15.9	1.06	6.33	0.04	18.00	3.86
− 78	15.8		5.00		20.35	5.54

water vapor than to those from dissociated hydrogen peroxide alone. Exact correspondence would not be expected; not only was the concentration of hydrogen atoms much lower than that present in dissociated water vapor but there was probably some reaction between hydrogen atoms and hydrogen peroxide (see later).

Reactions in Systems of Significance to the Formulation of a Mechanism

The dissociation products of an electrical discharge through hydrogen peroxide or water vapor may be composed of a number of chemical species, both molecules (e.g. hydrogen peroxide, water, hydrogen, and oxygen) and fragments of molecules, (e.g. hydroxyl radicals, hydrogen atoms, oxygen atoms, and perhydroxyl radicals). This large number of possible primary and secondary

products of the discharge could lead to many reactions, some of which might occur in the gas stream, others on the walls of the tubing between the discharge tube and trap, and still others in the cold trap itself. The investigation of certain reactions between some of these species is described below.

Addition of Molecular Hydrogen to Dissociated Peroxide

The reactants in this experiment were mixed in the large spherical reaction chamber (volume 5400 ml.). The hydrogen gas was supplied to this chamber at a steady rate (not through the discharge tube as in the previous experiment) and the products were collected in a trap cooled in liquid nitrogen. The input to the discharge was 7.4 mM. of hydrogen peroxide and 1.4 mM. of water per hour, while 16.4 mM. of hydrogen per hour was passed into the reaction chamber. The pressure in the discharge tube and reaction chamber was approximately 0.1 mm.

The products obtained when hydrogen gas was added to the dissociated peroxide vapor were identical with those from the dissociated peroxide itself. Therefore, the dissociation products of hydrogen peroxide must be unreactive toward molecular hydrogen.

Reactions of Dissociated Hydrogen Peroxide and Dissociated Water with Molecular Peroxide

In one series of experiments, the dissociated hydrogen peroxide vapor was mixed in a reaction vessel with molecular hydrogen peroxide and the products collected in a trap at − 195°C. The flow of molecular hydrogen peroxide into the reaction vessel was varied from 3.4 mM. per hour to 14.9 mM. per hour with a flow of 1.16 mM. of hydrogen peroxide and 0.64 mM. of water through the discharge tube. Sufficient helium gas was passed into the discharge to increase the operating pressure to about 0.2 mm. and to prevent back diffusion of molecular hydrogen peroxide into the discharge. Experiments were made at temperatures of 26°C. and 100°C. in the reaction chamber.

At low molecular peroxide flow rates, the amount of water produced in the reaction at 26°C. was directly proportional to the molecular hydrogen peroxide flow rate (Curve 2, Fig. 3), while at higher flow rates, the production of water became constant. In these experiments the peroxide found in the trap was assumed to be unreacted, an assumption supported by the absence of any oxygen evolution from the product. No significant change was found in the products of the reaction at 100°C.

The reaction observed between the dissociated hydrogen peroxide and molecular hydrogen peroxide could have been due, in part at least, to hydrogen atoms produced from the decomposition of the water which entered the discharge along with the peroxide. Since it was impractical to remove this water before the discharge, experiments similar to those outlined above were made by passing dissociated water vapor into molecular hydrogen peroxide to determine the relative effect of hydrogen atoms in the presence of hydroxyl radicals.

The input of water vapor to the discharge was 3.0 mM. per hour, which provided about the same theoretical production of OH radicals (3.0 mM. per hour) as before, with a fivefold increase in the production of H atoms (3.0 mM.

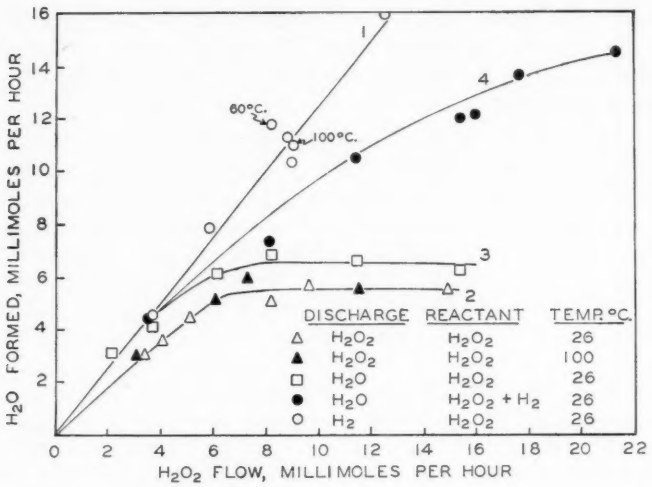

FIG. 3. Rate of production of water as a function of hydrogen peroxide flow.

as against 0.6 mM. per hour). Helium was again added to the discharge, for the reasons given previously. The temperature of the reaction vessel was 26°C. The input of molecular peroxide to the reaction vessel was varied from 2.15 to 15.42 mM. per hour.

The results obtained were similar to those obtained from the reaction of dissociated peroxide vapor with molecular peroxide. (Curve 3, Fig. 3). Again, the water production was a linear function of the amount of peroxide introduced at low flow rates, but became constant at higher flow rates. The yield became constant at about the same peroxide flow rate (6 mM. per hour), but was about 20% larger than the yield obtained from the reaction of dissociated hydrogen peroxide vapor at comparable flow rates.

Reaction of Hydrogen Atoms with Molecular Peroxide

Although only a relatively small effect of hydrogen atoms on molecular peroxide in the presence of a constant concentration of hydroxyl radicals was revealed in these experiments, the actual extent to which hydrogen atoms might attack peroxide was not evident. Experiments were therefore made in which molecular peroxide was subjected to the action of hydrogen atoms in the range of flow rates concerned.

Molecular hydrogen was introduced to the discharge tube which was operated at 1220 v. and 200 ma. at a pressure of about 0.25 mm. Wrede and thermocouple gauges were used to measure the hydrogen atom concentration, which was found to be about 10%. Since the molecular hydrogen flow into the discharge was about 16 mM. per hour, the production of hydrogen atoms was 1.8 mM. per hour. In two experiments, the reaction chamber was heated to 60°C. and 100°C.

The production of water from this reaction was a linear function of the

peroxide input at all flow rates studied (Curve 1, Fig. 3), in contrast to the constant value reached at high flow rates in both the dissociated water and dissociated peroxide reactions. In a study of the hydrogen atom – peroxide reaction at higher flow rates (12.0 to 37 mM./hr.) Geib (3) also found a linear relation between water formed and peroxide introduced.

At the higher reaction temperatures, no appreciable change in the product yield was observed.

The different behavior shown by this reaction could be the result of either the absence of hydroxyl radicals or the presence of excess molecular hydrogen. Experiments were therefore made to determine the effect of molecular hydrogen on the reaction of dissociated water vapor with molecular peroxide.

Reactions of Dissociated Water with Molecular Hydrogen and Peroxide

In these experiments molecular hydrogen was introduced into the reaction chamber at a rate of 18 mM. per hour, together with molecular hydrogen peroxide, while helium gas and 3.0 mM. per hour of water vapor entered the discharge tube. As before, the products of the reaction were collected in a trap cooled in liquid nitrogen.

Water formation from this reaction (Curve 4, Fig. 3) was intermediate between that from attack of peroxide by hydrogen atoms and that from the reactions of peroxide with dissociated peroxide or water vapors. An increase in temperature of the glass tubing at the entrance of the hydrogen peroxide and hydrogen mixture to the reaction vessel was observed, an effect found in the hydrogen atom, but not in the dissociated water and peroxide reactions with molecular peroxide.

DISCUSSION

The products obtained from dissociated hydrogen peroxide and dissociated water vapors exhibit much the same dependence on experimental conditions. The most obvious similarity, shown in Fig. 1, is the effect of trap temperature on the proportions of products. Both systems are capable of yielding products which evolve the same amount of oxygen on warming. Formation of hydrogen peroxide is observed in both systems at trap temperatures below − 120°C., and the temperature dependence of its formation is the same down to a trap temperature of − 170°C. The final products of the gas phase reactions in both systems are water, hydrogen, and oxygen. Further, an increase in the surface area to which the dissociated products are exposed leads to an increase in the amounts of oxygen and hydrogen from both systems.

It seems reasonable to assume that the close similarity in the behavior of the two systems is due to the presence of hydroxyl radicals in the dissociation products from both peroxide and water molecules. However it is unlikely that the similar behavior observed in the hydrogen atom – oxygen molecule reaction can be attributed to primary formation of hydroxyl radicals in that reaction. The mechanism postulated by Jones and Winkler as an adaptation of one proposed by Badin (1) appears to afford a satisfactory explanation of the experimental observations with all three systems. This mechanism assumes the formation of an unstable collision complex from either two OH radicals, or two H

atoms and an O_2 molecule. Stabilization of the complex by collision with a cold wall is assumed to produce either normal hydrogen peroxide or the abnormal form of peroxide postulated by Geib and Harteck, which is unstable above − 115°C., and decomposes into water and oxygen. If the complex is not stabilized, it may decompose either reversibly to the original reactants, or irreversibly to water and atomic oxygen. (The present authors have confirmed the report of Harteck and Kopsch (4), that oxygen atoms do not react with water vapor at room temperature or 100°C.)

On the other hand, Bawn has reported* thermochemical measurements made during oxygen evolution from the warmed products, and has expressed the opinion that the relatively small heat effect observed (*ca.* 300 cal.) is not consistent with decomposition of a molecular entity.

Fig. 1 shows that the yield of water below a trap temperature of − 120°C. and the yield of hydrogen peroxide below − 170°C. from dissociated water vapor were larger than the corresponding yields from dissociated hydrogen peroxide vapor. This behavior suggests that the following trap reactions occurred in the water vapor system where excess H atoms were present.

$$H + HO_2 \rightarrow H_2O_2 \text{ below } -170°C.$$
$$H + OH \rightarrow H_2O \text{ below } -120°C.$$

The products of the discharge through hydrogen – hydrogen peroxide mixture (Table III) showed the influence of these reactions.

If decomposition of an abnormal hydrogen peroxide is assumed to produce water, the amount of water so formed can be calculated from the amount of oxygen evolved. However, another source of water is apparent from the results in Fig. 2. In these experiments, a trap cooled in dry ice collected more water than a trap cooled in liquid nitrogen. Further, the difference in the yields was greatest at shorter reaction times. This suggests that formation of water occurred at a trap temperature of − 78°C., which possibly accounts for the rather large amount of water formed from dissociated hydrogen peroxide at − 78°C. with no reaction chamber (Fig. 1). This amount of water continued to be formed as the trap temperature was lowered, until formation of hydrogen peroxide became appreciable, after which a decrease in the amount of water produced by this means must have occurred. This behavior is readily explained on the basis of a complex which decomposes at trap temperatures down to those where it becomes stabilized to peroxide.

The surface reaction by which hydrogen and oxygen were formed is postulated to involve hydroxyl radicals as follows

$$OH + OH + M \rightarrow H_2 + O_2 + M.$$

The gas phase reactions of molecular peroxide with hydrogen atoms, (System I), dissociated peroxide, i.e. OH radicals (System II), and dissociated water, i.e. mixture of H atoms and OH radicals, in the presence (System III) and absence (System IV) of excess hydrogen are prohibited, in dissociated water vapor itself, by the absence of any molecular hydrogen peroxide. In view of the correspondence between the products of dissociated water vapor and hydrogen peroxide vapor (Fig. 1) these reactions are presumably unimportant in dissoci-

**Discussions of the Faraday Society, Toronto, Sept. 9, 1952.*

ated hydrogen peroxide vapor also. The decomposition of hydrogen peroxide in the discharge may then be considered to be fast compared with the reaction between hydroxyl and peroxide.

The results with Systems I to IV above can be explained on the basis of a chain mechanism, involving reactions of hydrogen atoms and hydroxyl radicals as follows:

$$H + H_2O_2 \rightarrow H_2O + OH \quad (1)$$
$$OH + H_2O_2 \rightarrow H_2O + HO_2 \quad (2)$$
$$HO_2 + H_2O_2 \rightarrow H_2O + O_2 + OH \quad (3)$$

This series of reactions is adequate to explain the mole for mole relation between water formed and hydrogen peroxide reacted in Systems II and III (Curves 2 and 3, Fig. 3) at all flow rates. To explain the constant amount of water formed at higher flow rates in these experiments, chain breaking reactions involving hydrogen peroxide as a third body appear to be necessary, viz.

$$OH + OH + [H_2O_2] \rightarrow H_2O_2 + [H_2O_2] \quad (4)$$
$$H + OH + [H_2O_2] \rightarrow H_2O + [H_2O_2] \quad (5)$$

Of all the species, H_2O_2 is apparently the most effective in accommodating the energy liberated in these reactions.

Curve 3 (Fig. 3) lies above Curve 2, presumably because of the relatively larger concentration of hydrogen atoms present, capable of initiating more chains by reaction 1. However the fact that a fivefold increase in the hydrogen atom concentration in this reaction over that which prevailed in System II caused an increase in water production of only 20% would seem to indicate that reaction 2 predominates. In the hydrogen atom reaction (Curve 1, Fig. 3) an additional step involving molecular hydrogen is possible

$$H_2 + HO_2 \rightarrow H_2O + OH \quad (6)$$

This reaction may explain the linear relation between water formed and input over the range of flow rates studied.

In addition to increasing the chain length, this reaction should lead to production of water in excess of the mole for mole amounts predicted by reactions 1, 2, and 3. The observed ratio in the hydrogen atom reaction was 1.3.

Reaction 6 also appears to be operative in the reaction between peroxide and dissociated water vapor in the presence of hydrogen (Curve 4). However the effect of reactions 4 and 5 is noticeable at higher flow rates.

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THE ALKALOIDS OF *LYCOPODIUM* SPECIES

XII. RELATIONSHIP BETWEEN SOME OF THE MINOR ALKALOIDS AND LYCOPODINE[1]

By Bryce Douglas,[2] D. G. Lewis,[2] and Léo Marion

ABSTRACT

Reduction of the carbonyl group of lycopodine with lithium aluminum hydride gives rise to dihydrolycopodine which contains a secondary alcoholic group. The new base can be dehydrated to form anhydrodihydrolycopodine and this is identical with alkaloid L14($C_{16}H_{25}N$). Acetylation of dihydrolycopodine produces O-acetyldihydrolycopodine which is identical with alkaloid L2($C_{18}H_{29}O_2N$). On distillation at atmospheric pressure alkaloid L2 is pyrolyzed and gives rise to 7-methylquinoline. Alkaloids L8 and L30 which had been reported previously as different have now been found to be identical.

Lycopodine ($C_{16}H_{25}ON$), the most widely distributed alkaloid in the *Lycopodium* species, contains a carbonyl group as indicated in its infrared absorption spectrum, and confirmed by the formation of a hydrazone and by conversion to a tertiary carbinol via the action of phenyl-lithium (5). It has now been possible also to prepare an oxime of lycopodine. The Beckmann rearrangement of this oxime, however, failed to take place under the usual conditions. Attempted condensation of lycopodine with benzaldehyde failed to give a benzylidene derivative. As previously reported the reduction of lycopodine with the aid of lithium aluminum hydride converted the carbonyl group to a secondary alcoholic group giving rise to dihydrolycopodine (5), a base which could be oxidized with chromic acid back to lycopodine.

Through the action of phosphorus pentachloride it was possible to remove the elements of water from dihydrolycopodine. The product of the reaction, anhydrodihydrolycopodine ($C_{16}H_{25}N$), was an oily base forming a crystalline perchlorate. Whereas the infrared absorption spectrum of dihydrolycopodine showed an absorption band at 3625 cm.$^{-1}$ indicative of a hydroxyl group, that band was absent in the spectrum of anhydrodihydrolycopodine. Alkaloid L14 which occurs in *L. tristachyum* (9) is isomeric with this base and gives rise to a perchlorate having the same optical rotation and the same melting point as anhydrodihydrolycopodine perchlorate. Admixture failed to alter the melting point and the X-ray diffraction patterns of both salts were superimposable. It can therefore be concluded that alkaloid L14 is anhydrodihydrolycopodine.

Dihydrolycopodine could be acetylated readily to O-acetyldihydrolycopodine, $C_{18}H_{29}O_2N$, which was crystalline and formed a crystalline perchlorate. The acetylated base was isomeric with alkaloid L2 occurring in *L. flabelliforme* (6). The infrared absorption spectrum of alkaloid L2 showed no absorption in the OH region, but contained a sharp absorption peak in the carbonyl region at 1730 cm.$^{-1}$ Further, the spectrum showed absorption in the region

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near 1239 cm.$^{-1}$ normally displayed by acetates. This absorption consisted of a double peak at 1228 and 1241 cm.$^{-1}$ The occurrence of a single maximum near 1239 cm.$^{-1}$ or of multiple peaks in this region has been related to cis–trans configuration (1, 4) or to the equatorial or polar orientation of the acetoxy group (2, 3). When the structure of lycopodine becomes better known this observation may prove useful. Both O-acetyldihydrolycopodine and its perchlorate had the same respective melting points as alkaloid L2 and its perchlorate, and admixture of the two bases and the two salts failed to alter these melting points. X-ray powder diagrams of the two perchlorates were exactly identical. Hence, alkaloid L2 is naturally occurring O-acetyldihydrolycopodine. Distillation of the acetylated base at atmospheric pressure brought about partial decomposition, the distillate consisting mostly of the unchanged material and a small quantity of an oily base which proved to be 7-methylquinoline. This supplied further evidence of the readiness with which lycopodine gives rise to 7-methylquinoline.

Alkaloid L8, $C_{15}H_{25}O_2N$, m.p. 180° (perchlorate, m.p. 318°), has been reported as occurring in *L. annotinum* (7). Later, alkaloid L30, m.p. 178°, forming a perchlorate melting at 311°, was found in *L. annotinum* var. *acrifolium* (8). On closer examination it has now been established that alkaloids L8 and L30 are identical, admixture of the bases and of their perchlorates failing to depress the respective melting points.

EXPERIMENTAL[3]

Lycopodine

Lycopodine had $[\alpha]_D^{26} -24.5°$ (*c*, 1.10 in absolute ethanol). Its pK, 7.44 (pH at half titration), was obtained by titration of the perchlorate in 50% methanol. The equivalent of the salt thus found was 347 (calcd. 347.4).

Lycopodine Oxime

Lycopodine (620 mgm.) dissolved in aqueous ethanol (20 ml.) was treated with molar hydroxylamine solution (2.5 ml.) and molar sodium acetate solution (3 ml.). The mixture was heated on the steam bath for three hours, cooled, and a drop of 10 *N* sodium hydroxide added. A solid was precipitated which was filtered and dried (307 mgm.), m.p. 220–230°. Recrystallization from aqueous ethanol gave colorless prismatic needles, m.p. 262–264°. Calcd. for $C_{16}H_{26}ON_2$: C, 73.25; H, 9.99; N, 10.68. Found: C, 72.74, 72.82; H, 9.77, 9.85; N, 11.02, 10.94%. The mother liquors yielded unchanged lycopodine (80 mgm.).

Dihydrolycopodine

This was prepared by the action of lithium aluminum hydride on lycopodine as previously described (5). It crystallized from ether as colorless prisms, m.p. 168°. Calcd. for one active hydrogen, 0.40; found, 0.44% (Zerewitinow). Its pK was 9.2 (value of pH at half titration of the perchlorate in 50% aqueous methanol), and the equivalent weight of the salt was 349 (calcd. 349.5). The infrared absorption spectrum of dihydrolycopodine contained no absorp-

[3] *All melting points are corrected.*

tion in the carbonyl region, but contained an absorption band at 3625 cm.$^{-1}$ indicative of a hydroxyl group.

Oxidation of Dihydrolycopodine

The base (94 mgm.) and chromic oxide (180 mgm.) were dissolved in purified glacial acetic acid (20 ml.) and the mixture maintained at 25° for six days. The brown solution was diluted with water (5 ml.), made slightly alkaline with sodium hydroxide, and extracted exhaustively with chloroform. The chloroform extract was washed repeatedly with 2*N* hydrochloric acid and the combined aqueous washings made alkaline with sodium hydroxide and extracted repeatedly with chloroform. This final combined chloroform solution was dried over anhydrous sodium sulphate and concentrated to yield an oil which solidified on seeding with a minute crystal of lycopodine (72 mgm.). After recrystallization the product melted at 116° either alone or after admixture with an authentic sample of lycopodine.

Anhydrodihydrolycopodine

Dihydrolycopodine (122 mgm.) and phosphorus pentachloride (200 mgm.) were dissolved in hot dry xylene (20 ml.) and heated under reflux in an atmosphere of nitrogen for six hours, during which time a considerable volume of hydrogen chloride was liberated. Water (20 ml.) was added to the cooled solution and the mixture shaken vigorously. The aqueous layer was separated and the xylene solution further extracted with three 20 ml. portions of 2 *N* hydrochloric acid. The combined acid extract was extracted once with a little chloroform, made slightly alkaline with sodium hydroxide, and extracted exhaustively with chloroform. This extract was dried and evaporated, leaving a brown syrup (103 mgm.) which was distilled *in vacuo*. A colorless oil was thus obtained, b.p. 125–130° at 1 mm., which slowly became yellow on standing. The infrared absorption spectrum of anhydrodihydrolycopodine in chloroform showed no hydroxyl absorption.

The bulk of the distilled oil was converted to the perchlorate in methanol by the usual procedure. The crystalline perchlorate separated from aqueous methanol as plates, m.p. 234–237°. Two recrystallizations from acetone yielded colorless prisms, m.p. 238–239°. Calcd. for $C_{16}H_{25}N.HClO_4$: C, 57.91; H, 7.90. Found: C, 57.94; H, 7.85%. $[\alpha]_D^{26} -107°$ (*c*, 1.10 in methanol). In admixture with the perchlorate of alkaloid L14 (m.p. 238°) the melting point of anhydrodihydrolycopodine perchlorate was unchanged. Alkaloid L14 perchlorate has $[\alpha]_D^{26} -105.5°$ (*c*, 1.86 in methanol). The X-ray diffraction patterns of both perchlorates were superimposable.

Acetyldihydrolycopodine

Dihydrolycopodine (200 mgm.), trifluoracetic anhydride (0.16 ml.), and acetic acid (0.1 ml.) were mixed and maintained at 25° overnight. The solution was poured into excess sodium bicarbonate solution, extracted exhaustively with ether, and the dried (anhydrous sodium sulphate) extract evaporated down to a pale yellow oil which solidified on scratching, m.p. 90–95°. This product was dissolved in methanol and converted to a perchlorate in the usual

way (yield 140 mgm., m.p. 240–242°). Recrystallization three times from methanol yielded colorless prisms, m.p. 246–247° when immersed at 205°. The melting point, however, varies with the rate of heating and the temperature of immersion. When heated from room temperature the melting point can be as low as 230°. Calcd. for $C_{18}H_{29}O_2N.HClO_4$: C, 55.15; H, 7.71; N, 3.57. Found: C, 55.10, 55.27; H, 7.72, 7.71; N, 3.37, 3.53%. The pK of the base determined by titration of the perchlorate in 50% methanol with 0.063 *N* sodium hydroxide was 8.4 (value of pH at half titration) and the equivalent weight of the salt was 390 (calcd. 392). Alkaloid L2 perchlorate (m.p. 231°) was isomeric with the perchlorate of acetyldihydrolycopodine and in admixture with it melted at 246–247° when immersed at 205°. X-ray diffraction patterns of the two perchlorates were superimposable.

Acetyldihydrolycopodine perchlorate was dissolved in the minimum volume of water, the solution made alkaline with ammonia and the precipitated white needles collected, washed with water, and dried, m.p. 95–96°. In admixture with alkaloid L2 (m.p. 97°) the base melted at 95–96°. The acetylated base was also obtained by the action of acetic anhydride on dihydrolycopodine. The infrared absorption spectrum of acetyldihydrolycopodine shows absorption bands at 1730 cm.$^{-1}$ in the carbonyl region and at 1238 and 1241 cm.$^{-1}$ in the "acetyl region."

A 5 *N* solution of sodium hydroxide in 50% methanol (2 ml.) was added to acetyldihydrolycopodine (50 mgm.) dissolved in methanol (3 ml.) and the solution maintained at 40–50° overnight. The bulk of the solvent evaporated during this time. The aqueous solution was cooled and the separated oil which solidified was collected, washed, and dried (43 mgm.), m.p. 160–163°. In admixture with an authentic specimen of dihydrolycopodine (m.p. 168°) it melted at 164–165°.

Thermal Decomposition of Acetyldihydrolycopodine

Acetyldihydrolycopodine (700 mgm.) was heated in a stream of nitrogen under the vacuum of a water-pump in a small distilling flask. Almost all of the compound distilled unchanged. The distillate was redistilled at atmospheric pressure. A first fraction was obtained which was slightly yellow (480 mgm.) and a second high boiling fraction (155 mgm.) consisted of a colorless oil with a strong quinoline-like odor. The first fraction was converted to a perchlorate in acetone–ether, m.p. 216–220°. Recrystallization from methanol gave colorless rhombs, m.p. 246–247° (immersed at 205°) which showed no depression when mixed with acetyldihydrolycopodine perchlorate. The high boiling fraction was dissolved in dilute hydrochloric acid and extracted with ether. The ether extract yielded only a small trace of oil which was discarded. The acidic solution was made alkaline with sodium hydroxide and extracted exhaustively with chloroform. The extract was dried over anhydrous sodium sulphate and distilled on the steam bath to remove the solvent. There was left an oil (20 mgm.) which was converted to a picrate in methanol. After recrystallization from methanol the picrate consisted of lemon-yellow needles, m.p. 235–236° either alone or in admixture with an authentic specimen of 7-methylquinoline picrate.

Alkaloids L8 and L30

Alkaloid L8 ($C_{16}H_{25}O_2N$) was reported as having m.p. 180° and forming a perchlorate, m.p. 318° (4). Alkaloid L30 ($C_{16}H_{25}O_2N$), m.p. 178°, formed a perchlorate, m.p. 311°. In admixture, the two bases had the same melting point and so had the two perchlorates.

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THE ESSENTIAL OIL OF *THUJA OCCIDENTALIS* L.[1]

By A. C. Shaw

ABSTRACT

Foliage of *Thuja occidentalis* yielded 0.269% of an essential oil containing *l*-thujone, *d*-isothujone, *l*-fenchone, *l*-bornyl acetate, *dl*-limonene, *d*-sabinene, *l*-β-pinene, *d*-terpinen-4-ol, *dl*-α-pinene, *l*-camphor, *l*-camphene, myrcene, *l*-α-thujene, and an unidentified ester.

INTRODUCTION

Thuja occidentalis or eastern white cedar ranges from the maritime provinces westward as far as Lake Winnipeg, and northward to the southern end of James Bay. The essential oil of *T. occidentalis* was examined by Wallach (8, 9) who reported it to contain *d*-α-pinene, *l*-fenchone, *d*-thujone, esters of acetic acid, and carvatanacetone. It was noted that, at elevated temperatures, thujone is isomerized to carvatanacetone (5), and that the latter might not have been present in the oil prior to fractional distillation. The presence of esters of valeric acid has also been reported (3).

The present contribution is the last of a series describing the oils which are obtainable in acceptable yield from coniferous species which are cut commercially in Canada.

EXPERIMENTAL

Preparation of the Oil

Leaves and terminal branches of *T. occidentalis*, collected in April 1948, were distilled for six hours with steam from a boiler at 90 lb. pressure. The oil, obtained in a yield of 0.269%, had the following properties: η_D^{20} 1.4612, d_{20}^{20} 0.9168, α_D^{30} −7.8°, acid number 0.53, ester number 33.7, ester number after acetylation 72.7, aldehydes as citral 1.4%, ketones as carvone 50.7%.

Fractional Distillation of the Oil

The oil was distilled through a 6-ft., 25-mm. I. D. Podbielniak column using a reflux ratio of 20/1, and a pressure drop across the column of 20 mm. The properties of the fractions obtained are listed in Table I.

Identification of Constituents

Melting points were measured with a Kofler micro hot stage apparatus. Optical rotations were measured in a 1-dcm. tube.

l-α-Thujene

An unidentified, low-boiling fraction, b.p. 96–133°C., η_D^{20} 1.4265, α_D +1.60°, and a levo compound, b.p. 150.5–152.5°C., η_D^{20} 1.4559, α_D −14.76°, were separated by redistillation of fraction 1. The latter compound (0.5 gm.) when treated with hydrogen chloride in ether solution at 0° yielded 0.09 gm. of terpinene hydrochloride, m.p. 50–51°C.; mixed with dipentene hydrochloride, m.p.

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TABLE I

Fraction	η_D^{20}	d_4^{20}	α_D	B.p.	% Distilled
1	1.4484	0.846	− 1.55°	140.8°/760 mm.	0.55
2	1.4600	0.842	− 10.79	155.0	1.17
3	1.4623	0.849	− 7.36	156.0	1.80
4	1.4641	0.850	− 5.47	156.5	2.43
5	1.4655	0.855	− 5.95	156.9	3.05
6	1.4676	0.855	− 10.04	157.7	3.68
7	1.4692	0.857	− 17.45	148.3	4.31
8	1.4708	0.860	− 28.95	159.2	4.92
9	1.4713	0.863	− 40.86	159.6	5.42
10	1.4709	0.857	− 6.88	161.7	6.05
11	1.4703	0.846	+ 55.53	164.5	6.75
12	1.4705	0.845	+ 55.47	165.2	7.36
13	1.4710	0.848	+ 51.25	165.3	7.98
14	1.4717	0.848	+ 43.95	165.5	8.69
15	1.4722	0.844	+ 31.07	165.8	9.32
16	1.4703	0.810	+ 6.83	168.0	9.89
17	1.4705	0.816	+ 5.14	170.5	10.46
18	1.4730	0.837	+ 3.54	171.5	11.02
19	1.4773	0.838	+ 4.51	176.0	11.65
20	1.4781	0.840	− 9.72	177.4	12.24
21	1.4785	0.840	− 11.34	177.8	12.75
22	1.4698	0.884	− 22.43	178.0	14.72
Nonvolatile residue					14.95
Loss					15.03
23	1.4556	0.922	− 32.44°	105.5°/50 mm.	38.54
24	1.4535	0.923	− 32.74	106	39.18
25	1.4511	0.918	− 26.01	109	41.82
26	1.4505	0.916	− 22.62	109	42.55
27	1.4503	0.915	− 21.78	109	45.18
28	1.4497	0.914	− 18.26		45.87
29	1.4493	0.913	− 16.89	90°/20 mm.	46.60
30	1.4493	0.912	− 17.27		49.24
31	1.4490	0.912	− 16.67		54.22
32	1.4488	0.912	− 14.09		62.14
33	1.4490	0.913	− 7.23		62.81
34	1.4490	0.913	− 6.33		63.45
35	1.4490	—	— 6.33		64.12
36	1.4486	0.912	− 5.46		64.81
37	1.4489	0.913	− 9.12	90°/20 mm.	65.49
38	1.4488	0.913	− 10.00		66.14
39	1.4487	—	− 6.97		66.81
40	1.4488	0.914	− 2.86		67.45
41	1.4490	—	+ 1.51		68.12
42	1.4490	—	+ 4.53		68.75
43	1.4490	0.914	+ 6.89		69.42
44	1.4490	—	+ 9.94		70.07
45	1.4490	—	+ 13.58		70.74
46	1.4490	0.914	+ 17.03		71.38
47	1.4490	0.915	+ 10.44		72.08
48	1.4492	0.915	+ 19.23		72.73
49	1.4494	—	+ 29.07		73.42
50	1.4494	0.914	+ 33.81		74.13
51	1.4494	—	+ 40.14		74.77
52	1.4494	—	+ 44.68		75.41
53	1.4496	0.915	+ 48.81		76.10
54	1.4495	0.914	+ 32.78		76.76
55	1.4495	0.917	+ 55.45		77.40
56	1.4495	0.917	+ 61.85		78.06
57	1.4494	0.916	+ 65.00		78.70
58	1.4504	0.913	+ 65.52	91°/20 mm.	79.37
59	1.4568	0.934	+ 22.70		79.95

TABLE I (Concluded)

Fraction	η_D^{20}	d_4^{20}	α_D	B.p.	% Distilled
60	Solid, m.p. 90–110°				81.78
61	1.4726	0.949	+ 5.51°	102°/20 mm.	82.44
62	1.4740	0.940	+ 22.91	102	83.12
63	1.4750	0.939	+ 19.40	102	83.69
64	1.4760	0.944	+ 8.39	108.5	84.42
65	1.4693	0.958	− 3.30	110	85.08
66	1.4634	0.967	− 11.83	110	85.79
67	1.4634	0.967	− 18.02	110	86.43
68	1.4632	0.968	− 22.32	110	87.13
69	1.4632	0.969	− 27.38	110	87.81
70	1.4650	0.961	− 26.44	98°/10 mm.	88.52
71	1.4650	0.960	− 26.34	102	89.27
72	1.4659	0.958	− 25.71	104	89.95
Nonvolatile residue					90.52
Sesquiterpenes and high boiling compounds					98.42

40°C.; mixed with terpinene hydrochloride prepared from an authentic sample of sabinene from oil of savin, m.p. 50–51°C.

A mixture of 1.0 gm. of the levo compound and 2.35 gm. of potassium permanganate in 100 ml. of ice water was shaken for 24 hr., the manganese dioxide was separated and washed, and the combined aqueous liquors extracted with ether, acidified, saturated with salt, and again extracted with ether. From the latter extract was obtained 1.1 gm. of a noncrystallizing oil. This oil on standing for several days with an ethanolic solution of semicarbazide formed a semicarbazone which crystallized from ethanol in short needles, m.p. 198–199° C., alone, or in admixture with an authentic sample of *l*-α-thujaketonic acid semicarbazone.

dl-α-Pinene

Fraction 5 (17 gm.) when oxidized with permanganate by the method of Delepine (2) yielded 11.2 gm. of crude acids. After two crystallizations from a benzene–hexane mixture, the product melted at 103–104° C., undepressed by a known specimen of *dl*-pinonic acid.

l-Camphene

Although no solid terpene was isolated, the high negative rotation of fraction 9 suggested the presence of a small amount of camphene. Five grams of the fraction was hydrated with Bertram–Walbaum reagent (1). The resultant ester was saponified with ethanolic caustic, and poured into water. The oily paste which separated, when triturated with cold hexane, yielded 2.1 gm. of crude isoborneol, identified as the *p*-nitrobenzoate m.p. 131–133° C. undepressed by a known specimen.

d-Sabinene and l-β-Pinene

Fraction 11 (5.0 gm.) in 10 ml. of ether was saturated with hydrogen chloride at 0° C., refrigerated overnight, freed of ether, and refrigerated for several days. Oils were removed from the partially crystalline product on a cold porous tile, leaving 1.6 gm. of a hydrochloride, m.p. 50–51° C., after three crystallizations

from ethanol. The melting point of a mixture of this derivative and the terpinene hydrochloride prepared from α-thujene was not depressed. Terpinene hydrochloride may be formed by the action of hydrogen chloride on α-thujene, sabinene, α-, β-, or γ-terpinene, or β-phellandrene, as well as terpinen-4-ol and terpinen-1-ol.

Fraction 11 (24.1 gm.) was shaken for one hour with 57 gm. of potassium permanganate and 12 gm. of sodium hydroxide in 400 ml. of water containing 400 gm. of crushed ice. The solution was freed from manganese dioxide in the centrifuge, once extracted with chloroform, concentrated to 300 ml., and refrigerated. The product (1.7 gm.)—a sparingly soluble sodium salt—was recrystallized from water. From an acidified solution of the salt, the free acid was extracted with ether, and crystallized from hexane. Surprisingly *l*-nopinic acid, m.p. 125–126° C., m.p. with a known sample 126–127° C., derived from *l*-β-pinene, was obtained. The terpinene hydrochloride could not have been formed from β-pinene.

The aqueous oxidation liquors were therefore acidified, and extracted with chloroform. The noncrystallizing oil thus obtained, when treated with 5 ml. of 10% aqueous sodium hydroxide, yielded a crystalline salt (0.08 gm. of white platelets after two crystallizations from water). The free acid, isolated as before and crystallized from hexane and then from water, gave a few milligrams of sabinenic acid, m.p. 56–57° C. alone, or in admixture with an authentic sample prepared from oil of savin sabinene.

Myrcene

Fraction 16 (2.7 gm.) reacted violently when warmed with 2.0 gm. of maleic anhydride. Crude adduct (2.6 gm., b.p. 190–200° C. at 10 mm.) was separated by distillation, and crystallized from hexane. The product melted at 35–36° C. alone, or in mixture with *cis*-4-isohexenyl-Δ^4-tetrahydrophthalic anhydride.

dl-Limonene

Bromine (ca. 7 ml.) was added dropwise to an ice-cold solution of 10 gm. of fraction 21 in 10 ml. of isoamyl alcohol and 20 ml. of absolute ethyl ether. On evaporation of the ether 8.7 gm. of bromide separated and was recrystallized from ethanol. *dl*-Limonene tetrabromide, m.p. 125–126°C., undepressed by an authentic sample, was obtained.

l-Fenchone

Fraction 23 was redistilled and a compound with optimum properties, η_D^{20} 1.4620, d_4^{20} 0.9406, α_D − 55.66°, b.p. 103° at 50 mm., comprising 7.8% of the oil obtained. A part of this material was heated with three volumes of nitric acid until brown fumes were no longer evolved, washed, steam distilled, dried, and made to solidify in dry ice. The melting point, measured with an immersed thermometer, was 5.0°C. Fenchone melts at 5–6°C. and is stable to nitric acid.

A solution of 5.0 gm. of the fraction with 11 gm. of hydroxylamine hydrochloride and 6 gm. of potassium hydroxide in 80 ml. of ethanol in 10 days at room temperature deposited 1.21 gm. of beautifully crystalline oxime, m.p. 165–167° C., $[\alpha]_D$ − 44.0° (c = 3.03, ethanol). Hückel and Sachs (4) for *d*-fenchone-α-oxime reported m.p. 167°C., $[\alpha]_D$ + 46.5° (96% ethanol).

l-Thujone and d-Isothujone

Fractions 26 to 58 were mixtures of two compounds difficultly separable by

distillation. From fraction 32 was prepared a semicarbazone, m.p. 197–199° C., $[\alpha]_D$ + 53.7° (c = 1.02, ethanol), which must have been *l*-thujone semicarbazone, since it was decomposed by the action of oxalic acid and steam to yield an oil η_D^{20} 1.4490. This oil (0.4 gm.) was oxidized by 40 ml. of cold aqueous 2% potassium permanganate to α-thujaketonic acid (0.19 gm.) m.p. 74.5–75.5° C., $[\alpha]_D$ + 173.4° (c = 1.04, ether).

Fraction 53 semicarbazone melted at 170–175° C., $[\alpha]_D$ + 180° (c = 2.49, chloroform). For *d*-isothujone semicarbazone Short and Read (6) reported m.p. 172° C., $[\alpha]_D$ + 222° (c = 1, methanol). The ketone, regenerated from the semicarbazone as before, had η_D^{20} 1.4494, d_4^{20} 0.916, α_D + 66.3°, and was oxidized by permanganate to α-thujaketonic acid.

l-Camphor

The solid from fraction 60 melted at 90–110° C., $[\alpha]_D$ − 35.5° (c = 2.3, ethanol). It formed a 2,4-dinitrophenyl hydrazone (0.37 gm. from 0.5 gm.), m.p. 175–176° C., undepressed by *l*-camphor 2,4-dinitrophenyl hydrazone.

d-Terpinen-4-ol

When fraction 62 was treated with hydrogen chloride as previously described, a small amount of terpinene hydrochloride, m.p. 50–51°, was obtained. This derivative could have been formed from terpinen-1-ol, terpinen-4-ol, or possibly γ-terpineol. The oil (3.75 gm.) was shaken with an ice-cold solution of 2.55 gm. of permanganate in 200 ml. of water, and refrigerated overnight. The aqueous liquors were freed of manganese dioxide, neutralized with carbon dioxide, extracted with a small volume of ether, and finally evaporated to dryness. The residue was exhaustively extracted with ethanol, the ethanol removed in a vacuum desiccator, and the ethanol-soluble residue exhaustively extracted with benzene. On cooling, the glycerol separated as a gelatinous precipitate, which was purified by repeated crystallization from chloroform. The product did not melt sharply, but rectangular anisotropic needles melting up to 128° C. were obtained. *p*-Menthane 1,2,4-triol melts at 128–129° C. The glycerols derived from terpinen-1-ol and γ-terpineol melt at 120–121° C. and 110–112° C. respectively.

Bornyl Acetate

Fraction 69 was saponified, the alcohol fragment identified as the *p*-nitrobenzoate, and the acid fragment converted to the anilide, as previously described (7). *l*-Borneol, m.p. 204–206° C., *p*-nitrobenzoate, m.p. 135–136° C., $[\alpha]_D$ − 37.8° (c = 4.53, chloroform), and acetanilide m.p. 114° C., undepressed by an authentic sample, were obtained.

Fractions 18 and 19

The dextrorotation of these fractions cannot be due to contamination with *d*-sabinene, since their boiling points differ from that of *d*-sabinene by more than 6° C. Fraction 19 (1.0 gm.) in 2 ml. of ether, when treated with hydrogen chloride, gave 0.13 gm. of *dl*-limonene hydrochloride m.p. 50° C. Fraction 18, treated in the same manner, gave 0.06 gm. of a hydrochloride, m.p. 71.5–72° C., which may have been sylvestrene hydrochloride. No authentic specimen was available. Both fractions failed to form crystalline nitrosites or nitrosates, indicating the absence

of Δ^3-carene, α- or β-phellandrene. These fractions may contain Δ^4-carene.

Higher-boiling Compounds

The fraction of cedar oil boiling above 110° C. at 10 mm. (7.9% of the oil) was fractionally distilled through a 1-ft., 25-mm. I.D. Podbielniak column at 10 mm. pressure, 3 mm. back pressure, and reflux ratio 15/1. Sixty fractions were collected. The plot of properties against weight distilled indicated that at least 10 constituents were present but only five had been isolated in a state of approximate purity. The properties of these fractions are listed in Table II.

TABLE II

Fraction	η_D^{20}	d_4^{20}	α_D	Boiling range, °C.	% of original oil
A	1.4655	0.962	− 21.84°	96.4–98.2° (10 mm.)	0.5
B	1.4645	0.962	− 38.91	105.8–109.4 (10 mm.)	0.6
C	1.4955	0.968	+ 17.7	116–124 (10 mm.)	0.4
D	1.4888	0.983	− 35.4	115.3–116.2 (2 mm.)	0.1
E	1.5033	1.026	—	159.3–161.0 (1 mm.)	0.1

Fraction *A* was *l*-bornyl acetate. Fraction *B* was an ester which on saponification yielded acetic acid; *s*-benzyl thiuronium salt m.p. 133° C.; Ducleaux constants 6.8, 7.1, and 7.4. The alcohol portion (η_D^{20} 1.4748, d_4^{20} 0.935, α_D − 49.62°) formed a *p*-nitrobenzoate (0.29 gm. from 0.75 gm.), m.p. 140–141.3° C., $[\alpha]_D$ − 38.1° (c = 5.67, chloroform), but was dehydrated by phenyl isocyanate, and gave only oily products with hydrogen chloride.

Fraction *C* (0.7 gm.) when dehydrogenated with palladium–charcoal and treated with picric acid gave 0.08 gm. of cadalene picrate m.p. 115–117° C., but yielded no crystalline derivatives with *p*-nitrobenzoyl chloride or phenyl isocyanate. At 100° C. it reacted violently with sodium, but below 70° C. the reaction proceeded sluggishly or not at all. Fraction *C* may have contained an oxygenated derivative of a sesquiterpene related to cadalene. However, the yield of cadalene was low and the purity of the fraction questionable.

Fractions *D* and *E* yielded no aromatic products on dehydrogenation. Too little material was available to permit more detailed examination of these fractions.

RESULTS

From the data of Table I, a plot of properties against per cent distilled was made, and inflection points in the curve representing each property were marked. The per cent occurrence of each compound was given by the distance between the inflection points of the corresponding plateau. The percentage composition of the oil is as follows.

Terpenes		Oxygen compounds	
dl-Limonene	3.6%	*l*-Thujone	47.5%
d-Sabinene and *l*-β-pinene	3.5%	*d*-Isothujone	9.2%
dl-α-Pinene	2.6%	*l*-Fenchone	7.8%
l-Camphene	2.0%	*l*-Bornyl acetate	5.9%
Δ^4-Carene ?	1.3%	*d*-Terpinen-4-ol	2.7%
Myrcene	0.9%	*l*-Camphor	2.5%
l-α-Thujene	0.8%	Unidentified ester b.p. 105.8–109.4° C. at 10 mm.	0.6%
Unidentified liquid b.p. 96–133° C. at 760 mm.	0.4%		
Sesquiterpenes and higher-boiling compounds	6.8%		

ACKNOWLEDGMENTS

This investigation was undertaken at the suggestion of Mr. R. N. Johnston, Chief, Division of Research, Department of Lands and Forests, Province of Ontario, and his colleagues, to whom we are also indebted for supplies of plant material. The essential oil was prepared by Mr. J. N. Brown and Dr. T. F. West. The investigation was supported by a grant from the Research Council of Ontario.

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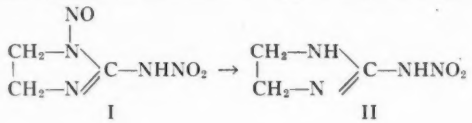
REACTION OF METHYLAMINE WITH 1-NITROSO-2-NITRAMINO-2-IMIDAZOLINE[1]

By A. F. McKay

ABSTRACT

On the addition of methylamine to 1-nitroso-2-nitramino-2-imidazoline, 2-nitramino-2-imidazoline, N-methyl-N′-nitroguanidine, N,N′-dimethyl-N″-nitroguanidine, and 2-methylamino-2-oxazolidine were obtained. This reaction is discussed briefly.

In a continuation of the studies (3, 4, 6) of the reactions of amines with 1-nitroso-2-nitramino-2-imidazoline (I) (6) methylamine was used. The order of addition in the latter reaction was reversed. This resulted in the denitrosation of part of the 1-nitroso-2-nitramino-2-imidazoline to give 2-nitramino-2-imidazoline (II) (7). The other products obtained, which are normal (4) to this type of reaction, were N-methyl-N′-nitroguanidine (III) (1), N,N′-dimethyl-N″-nitroguanidine (IV), and 2-methylamino-2-oxazolidine (V). The formation of these products and similar types of products from the reaction



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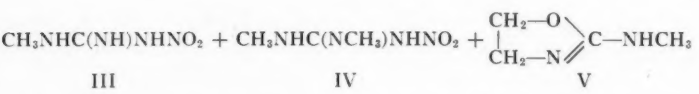

III IV V

of other amines with nitrosamides can be explained by the assumption of a carbonium ion intermediate (3, 5). However it should be emphasized that the mechanisms suggested (3, 5) are only offered as a method of describing a course for these reactions and predicting the reaction products from any given reaction in this series.

The structure of 2-methylamino-2-oxazolidine (V) was established by the cyclization of N-β-chloroethyl-N′-methylurea to this same compound by the method of Gabriel and Stelzner (2).

EXPERIMENTAL[2]

Reaction of Methylamine with 1-Nitroso-2-nitramino-2-imidazoline

Thirty grams (0.177 mole) of 1-nitroso-2-nitramino-2-imidazoline were suspended in 61 cc. of water and the mixture was maintained at 5–7° C. while 44 cc. of a 25% aqueous methylamine solution was added over a period of five minutes. After gas evolution had ceased, which required two hours and ten minutes, the solid was removed by filtration, yield 3.1 gm. (12.6%).

[1] *Manuscript received October 20, 1952.*
Contribution from Defence Research Chemical Laboratories, Ottawa, Ontario. Issued as D.R.C.L. Report No. 112.

[2] *All melting points are uncorrected. Microanalyses by Mr. C. W. Beazley, Skokie, Illinois.*

This solid melted at 220–221° C. with decomposition alone and on admixture with an authentic sample of 2-nitramino-2-imidazoline (7).

The filtrate was transferred to a crystallizing dish and on evaporation deposited a second crop of crystals (m.p. 143–151° C.), yield 2.82 gm. After several fractional crystallizations from 95% ethanol, these crystals were divided into the previously known N-methyl-N′-nitroguanidine (1) (380 mgm.) and N,N′-dimethyl-N″-nitroguanidine (100 mgm.). The remaining crystals appeared as a constant melting mixture (m.p. 147.5–148° C., hazy 145° C.). N-Methyl-N′-nitroguanidine (m.p. 160–160.8° C.) was identified by a mixed melting point determination with a known sample of methylnitroguanidine (m.p. 160–161° C.). The pure N,N′-dimethyl-N″-nitroguanidine melted at 170.5–171° C.

Anal. Calcd. for $C_3H_8N_4O_2$: C, 27.28; H, 6.06; N, 42.42%.
Found: C, 27.54; H, 6.28; N, 42.60%.

The filtrate from the second crop of crystals was treated with an aqueous saturated picric acid solution. The crystalline picrate melted at 149–157° C., yield 5.28 gm. (8.8%). Several crystallizations from 95% ethanol raised the melting point to 167° C. This picrate was identified as 2-methylamino-2-oxazolidine picrate by analysis and synthesis.

Anal. Calcd. for $C_{10}H_{11}N_5O_8$: C, 36.48; H, 3.34; N,21.29%.
Found: C, 36.58; H, 3.60; N, 21.44%.

Methyl Isocyanate

Methyl isocyanate (b.p. 34–36° C.) was prepared in 48% yield by the method of Slotta and Lorenz (8).

N-β-Chloroethyl-N′-methylurea

β-Chloroethylamine hydrochloride (15.90 gm.) was converted to the free amine in benzene solution as previously described (3). To this benzene solution, 5.45 gm. (0.096 mole) of methyl isocyanate was added under cooling. The benzene solution was cooled in the refrigerator until precipitation appeared complete. The solid (m.p. 65–70.0° C.) was recovered by filtration and washed with petroleum ether (b.p. 30–60° C.). Yield 13.1 gm. (99.8%). Several crystallizations from benzene – petroleum ether were required to obtain a constant melting point of 95–96° C.

Anal. Calcd. for $C_4H_9ClN_2O$: C, 35.19; H, 6.61; N, 20.52; Cl, 25.95%.
Found: C, 34.90; H, 6.63; N, 20.40; Cl, 25.30%.

2-Methylamino-2-oxazolidine Picrate

One gram (0.007 mole) of N-β-chloroethyl-N′-methylurea was refluxed with 10 cc. of water for 10 min. The homogeneous solution on addition of an aqueous solution of triethanolamine picrate solution gave 2.06 gm. (88.0%) of picrate (m.p. 164–166° C.). Two crystallizations from water did not alter the melting point. This compound did not depress the picrate (m.p. 166–167° C.) obtained from the reaction of methylamine with 1-nitroso-2-nitramino-2-imidazoline.

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ELECTROPHORESIS OF RESIN EMULSIONS

I. THE EFFECT OF EMULSIFYING AGENTS[1]

By L. A. Munro and F. H. Sexsmith

ABSTRACT

A modified Briggs electrophoretic glass cell was used to measure the mobility of over 2500 particles of vinyl acetate latices prepared with different emulsifying agents. Although anionic and cationic agents conferred negative and positive mobilities respectively, nonionic agents invariably resulted in negatively charged particles. This is attributed to partial chemical change to anionic materials or to hydrogen bonding and polarization processes. The nature of the emulsifying agent and electrolyte concentration rather than concentrations of the latex or particle size were the most significant variables affecting the mobility of any latex.

During the past ten years there has been rapid progress in the manufacture and use of aqueous suspensions of synthetic polymers (14). The dispersion of monomers to be polymerized in the emulsion state is effected by the use of surface-active agents. The latter are numerous, of a variety of structures and types, and are used either singly or in combinations. Variation in wetting agent or protective colloid in latex formulation alters the electrical charge on the disperse phase. Current commercial latices of good stability are cited as being negatively or positively charged. Some are described as neutral, although as will be shown, emulsions fitting this category are probably nonexistent. No quantitative data on particle charge or stability are available.

The following paper reports electrophoretic studies of polyvinyl acetate latices emulsified in the presence of various cationic and nonionic wetting agents and with combinations of these.

APPARATUS AND METHODS

Electrophoresis was measured using the microscope technique. Abramson has cited several advantages of this method (1).

Various microcells for electrophoresis have been described. Rectangular or flat cells have been used by several workers (4, 5, 6, 7, 8). Cells of cylindrical cross section have been preferred by others (2, 11, 12). Both types have inherent advantages and disadvantages.

The apparatus used for these studies embodied features of different cells described in the literature (2, 5, 6).

Pyrex capillary tubing was ground flat on both sides to within as small a distance from the actual bore as was possible (about 0.1 mm.). Four grades of abrasive on rotary-powered plates served to accomplish this grinding:

Carborundum F,
Emery 5 or Alundum 500,
Alundum 600,
Chrome oxide.

A micrometer was used to keep the sides as closely parallel as possible during this operation. Several capillary sections of varying radii were ground and polished.

[1] *Manuscript received October 23, 1952.*
Contribution from Department of Chemistry, Queen's University, Kingston, Ontario.

They were checked with the microscope to enable the tube of proper radius to be selected.

The ground and polished mid-section which was ultimately chosen was fused onto capillary tubing of approximately the same internal radius. The unground capillary was notched first to receive the thin cell, and very fine Pyrex rod was laid on either side of this recess (Fig. 1). The fine rod was fused and sealed into

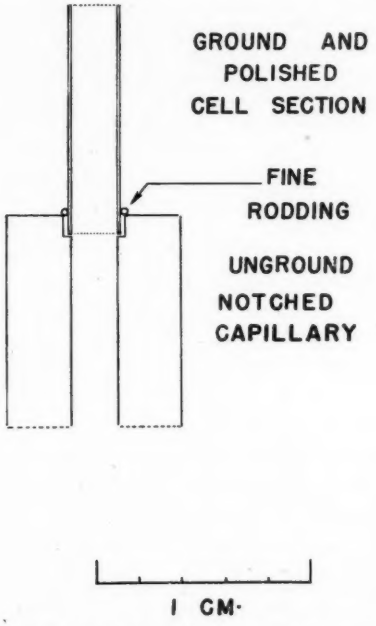

FIG. 1. Method of joining flat mid-section of cell to capillary tubing.

the recess with a small, cool flame, then stronger heating around the unground capillary end completed the operation. The complete cell is shown in Fig. 2.

The capillary used for the central optical section was of radius 1.087 mm. When a microscope equipped with a 47× air objective and 20× hyperplane eyepiece was focused on this central section, polyvinyl acetate emulsion particles contained therein were suitably magnified with a minimum of image distortion. Sufficient working distance was available for particles to be brought into focus anywhere from the roof of the bore to within half of the distance between the top and the axis. The cell itself could be completely cleaned by passage of liquid through it without removing it from the permanent microscope stand.

A potential across the cell of at least 200 v. was necessary to give the emulsion particles measurable electrophoretic velocities. A power pack, enabling 110 v. a-c. to be converted to 360 v. d-c., was used in conjunction with potentiometers and resistances for varying the potential down to zero. As shown in Fig. 3, the selective sensitivity of the potentiometers was increased for the upper voltage

range by inserting the 25,000 ohm resistance into the circuit. Under conditions of low salt concentration (less than 0.01 *M* as sodium chloride) the mercury electrodes were completely reversible. Currents through the cell never exceeded 1000 microamperes and generally were less than 50.

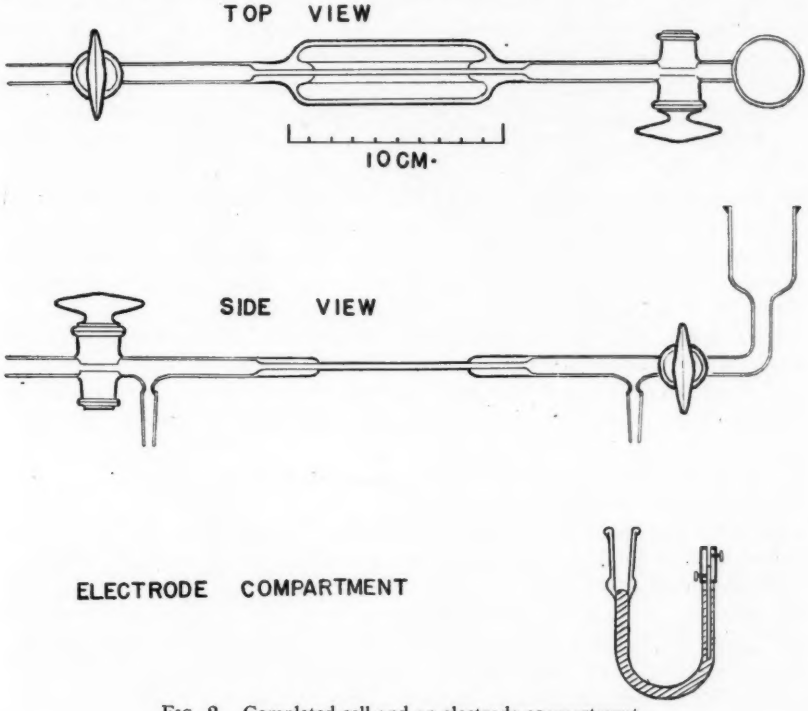

FIG. 2. Completed cell and an electrode compartment.

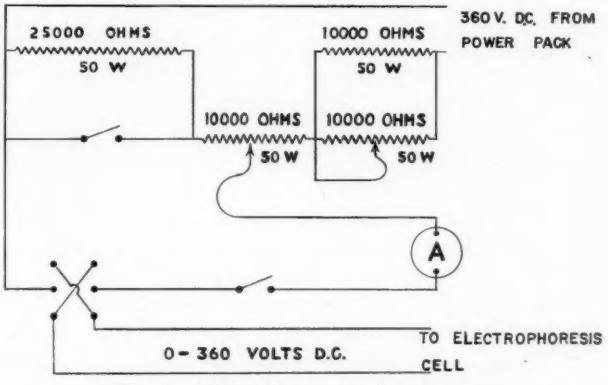

FIG. 3. Wiring diagram for the apparatus.

The use of reversible mercury electrodes offers advantages over electrode compartments previously designed for microelectrophoresis cells (2, 5, 6). These have combined reversible systems such as $Hg–Hg(NO_3)_2$, Ag–AgCl, and $Cu–CuSO_4$ with plugs of plaster or glass wool for hindering diffusion. With such arrangements it seems quite probable that minute changes in electrolyte concentration must take place in the sample for investigation once it is placed in the cell. Any small variation in salt concentration would introduce error into mobility measurements, especially when electrophoresis is being conducted at provisionally low ionic strengths. Data and discussion of this field will appear in a subsequent paper.

The stage of the microscope was removed to accommodate the central section of the cell. The complete cell was clamped securely to three small retort stands. By means of two screw pinions attached to the bench and pressing down and in from either side against the base of the microscope, the latter was made movable with respect to the cell. Turning these screws a fraction of a revolution made it possible to shift the entire microscope through a few thousandths of a centimeter across the bench top. The layout of the optical bench is shown in Fig. 4*A*.

The lighting arrangement was composed of a parabolic microscope light as source with a plane mirror inclined at 45°. Vertical reflected light thus struck the cell perpendicular to the flat sides. To determine the location of a particle in the cell visible through the microscope, the following correction was applied:

$$r = r_0 - n_w d.$$

r = vertical distance from cell bore to particle level.
r_0 = radius of capillary.
n_w = index of refraction of dispersion medium (water).
d = vertical displacement (air) from internal cell roof to particle level.

The fine adjustment of the microscope was calibrated into fiftieths of a turn to enable accurate measurement of d. One division = 4.82×10^{-3} mm. A graticule was sealed into the 20× hyperplane eyepiece. A particle traversing the full scale length moved through 140.3 microns.

The field strength (volts per cm.) in the center chamber

$$E = \frac{I}{q\sigma}.$$

E = field strength, σ = specific conductance of suspension,
I = current, q = cell constant.

Then:

$$\text{Electrophoretic mobility} = \frac{\text{particle velocity}}{E} \text{ (microns per sec. per volt per cm.)}$$

It has been established that with cells of this type, a considerable change of field strength occurs in experiments carried out over a wide range of ionic strengths, even though the applied voltage is constant (3). Hence it is not sufficient to measure the applied voltage alone and to assume that the drop in the cell is a constant fraction of the total drop. This important factor often has been ignored in earlier published work (13). With the apparatus as shown in Fig. 3 the current was conveniently kept constant during mobility measurements.

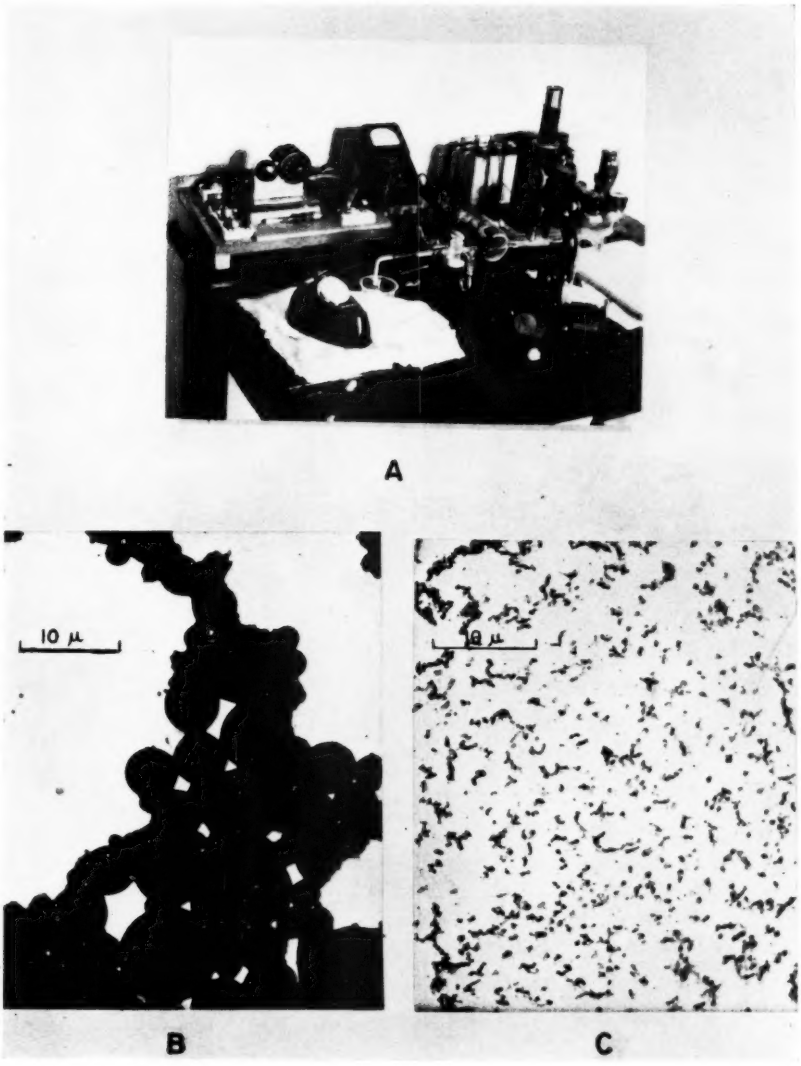

FIG. 4. *A*. General view of the apparatus.
B. Electron micrograph of latex (5CN) having the largest particle size of emulsions studied.
C. Electron micrograph of latex (17A) having the smallest particle size.

The actual cross-sectional area of the central capillary may be used as q, the cell constant, provided it is very small in comparison with the cross-sectional area of the end electrode compartments. Since this proviso did not hold for the cell in Fig. 2, calibration was carried out according to the quartz powder method of Smith and Lisse (12). This gave a value of 0.058.

The accuracy of this standardization method leaves much to be desired. Any trace of electrolyte present in the quartz suspension drastically affects the apparent electrophoretic mobility. However the experimental results are strictly comparable in spite of possible error introduced by the cell constant.

In determining the electrophoretic mobility of any sample, all measurements may be taken and averaged at the level $r_0/\sqrt{2}$ from the axis, or a plot of r^2/r_0^2 against velocity may be made, and the observed velocity where $r^2/r_0^2 = 0.50$ noted. (In the quartz standardization both methods were used.)

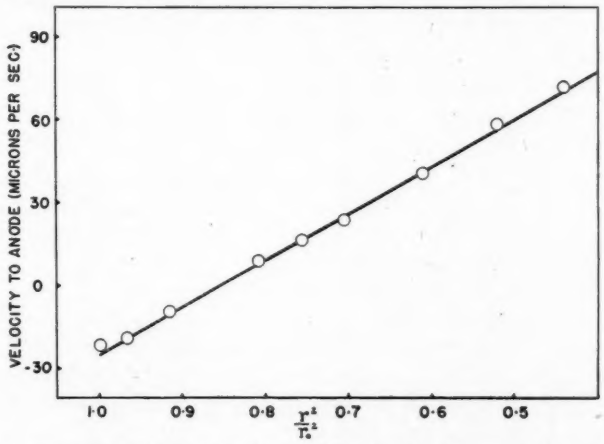

FIG. 5. Electrophoretic velocities of standard latex for different values of r^2/r_0^2.

Fig. 5 shows the straight line resulting when r^2/r_0^2 was plotted against velocity for a typical polyvinyl acetate emulsion. The depth of zero electrosmosis for single level measurements $r = 0.707\ r_0$ and $r = r_0 - n_w d$, where r = radius of capillary = 1.087 mm. The velocities of electrophoresis of a typical run when $r^2/r_0^2 = 0.50$ and from a determination at constant depth on the same resin dispersion give mobilities which agreed to 2%. The respective values are 3.40 and 3.33 microns per sec. per volt per cm. It was found most practical to conduct all routine measurements at the fixed level of focus only.

All samples for electrophoresis were brought to $20 \pm 0.3°$ C. The cell was rinsed with the diluted latex sample several times before electrophoresis, and periodically between readings to maintain constant temperature and electrolyte concentration in the cell.

Less significance may be attached to temperature control than to other variables. It has been shown that the mobility of a colloidal granule increases with the temperature of the suspension at about the same rate as the conductance of

the aqueous electrolyte increases or the viscosity of water decreases—about two per cent per degree centigrade near room temperature (13). The errors are mutually compensating.

Heating inside the cell due to electrode processes was nonexistent owing to the extremely low amperage used. However, it was found that noticeable convection currents were brought about by the heat of the light source. These were minimized by shutting off the lamp except when actual timed observations were being made. By reversing the double pole throw switch (Fig. 3) on alternate readings any convection error still present was completely eliminated. In practice it was found that the average of 20 readings, 10 in each direction of current flow, was sufficient to give reproducible results for different samples of the same emulsion. Powney and Wood (9) used a similar technique with a microscope cell for determining the mobility of oil drops in detergents and soap solutions. The necessity of taking as many as 20 readings is not due to intrinsic inefficiency of the apparatus, but rather to slight variation in charge from one droplet to another in the same sample.

Figs. 4*B* and 4*C* show the range of particle sizes for latices which were subsequently studied. As well as being illustrative of the diameter extremities which were encountered, they also exemplify different particle size distributions.

EFFECT OF DILUTION

Dilution of the samples to 0.01–0.25% total solids was made using double distilled water. For emulsions with large particle size the higher concentration was used whereas for fine emulsions concentrations were kept at less than 0.10% to avoid opacity in the central observation chamber.

In order to investigate the resultant effect on mobility, one anionic and one cationic emulsion were measured electrophoretically at varying percentage of solids. Each determination was carried out at constant specific conductance. That the differences in concentrations used have no effect on the mobility is shown in Figs. 6 and 7.

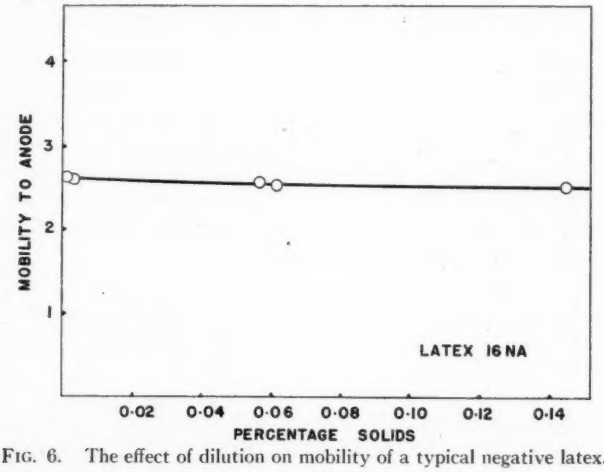

FIG. 6. The effect of dilution on mobility of a typical negative latex.

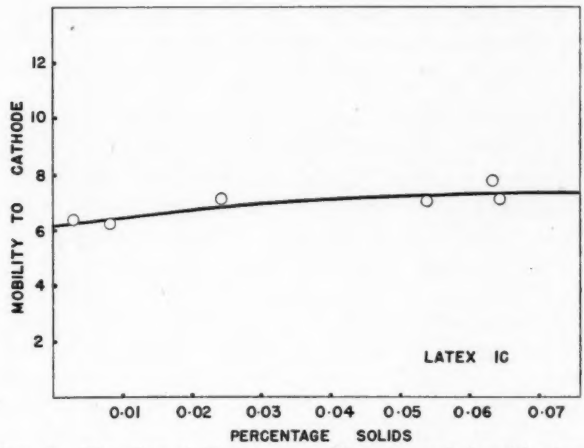

FIG. 7. The effect of dilution on mobility of a typical positive latex.

HYDROGEN ION CONCENTRATION

The pH of the diluted latices varied from 4–6. In order to evaluate this effect samples of representative diluted emulsions of the same percentage solids were adjusted with hydrochloric acid, sodium chloride, or sodium hydroxide to different pH values, the specific conductance being made equal to that of $2.57 \times 10^{-4} M$

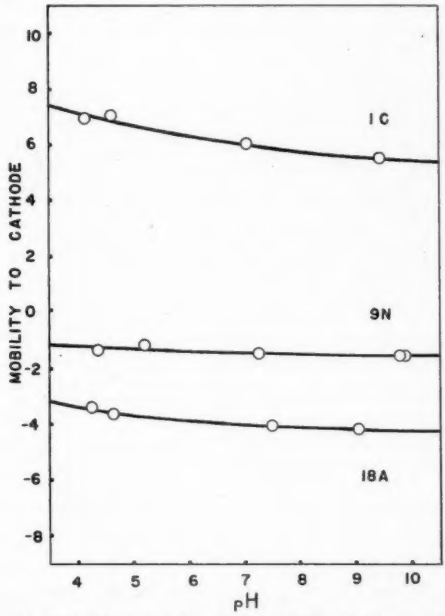

FIG. 8. The effect of pH on the mobility of latex particles.

sodium chloride in each case. The results indicate that the change in mobility over the pH range studied is very slight (Fig. 8).

STANDARD MOBILITIES

Table I lists the standard mobilities of a series of emulsions. The standard values are for latices containing 0.01–0.10% solids, electrolyte concentration $= 5.00 \times 10^{-5} M$ sodium chloride, and pH as obtained on dilution with addition of dilute sodium chloride to reach standard conductance. The designation (A) indicates an emulsifier of the anionic type, (C) a cationic type, (N) a nonionic agent.

TABLE I

Latex	Emulsifying agents or protective colloids	Absolute mobility, μ/sec./v./cm.	pH	Average particle diameter, microns	Particle size variation*	Particle charge variation
1C	Octadecyl-, hexadecyl-, dodecyldimethyl ammonium chloride and $C_{18}H_{37}NH-P(=O)(OR)(ONH_3C_{18}H_{37})$	5.28	4.4	1–5	3	2
2C	Cetyldimethylbenzyl ammonium chloride	4.29	5	1–5	3	1
3C	Paradiisobutylphenoxyethoxyethyl-dimethylbenzyl ammonium chloride	3.83	5	1–5	3	1
4CN	Cetyldimethylbenzyl ammonium chloride and polyvinyl alcohol	2.38	5	1–5	2	1
5CN	Cationic and nonionic	1.54	5.5	5–10	3	2
6CN	Hydroxyethyl cellulose and paradiisobutylphenoxyethoxyethyldimethylbenzyl ammonium chloride	0.49	5	0–2	3	1
7N	Nonionic	− 0.49	6.3	1–5	2	3
8N	Polyvinyl alcohol	− 1.27	5.5	1–5	1	2
9N	Polyvinyl alcohol	− 1.35	5.5	1–5	3	2
10NA	Anionic and nonionic	− 1.52	6.1	0–2	1	1
11N	Gelatin	− 1.58	4.8	0–2	1	2
12N	Nonionic	− 1.89	5.7	5–10	1	1
13N	Hydroxyethyl cellulose	− 2.30	5.3	0–1	0	1
14NA	Polyvinyl alcohol and gum tragacanth	− 2.80	5.2	5–10	2	1
15NA	Polyvinyl alcohol and gum tragacanth	− 2.90	4.9	1–5	2	1
16NA	Polyvinyl alcohol and gum tragacanth	− 2.96	5.2	1–5	3	1
17A	Anionic	− 3.10	5–6.5	0–2	1	1
18A	Gum arabic	− 3.30	5.3	0–2	1	1
19A	Gum arabic	− 3.32	6.0	0–2	1	1
20NA	Gum tragacanth and polyvinyl alcohol	− 3.32	5.4	5–10	2	1

* *0—Uniform. 1—Slight variation. 2—Moderate. 3—Considerable variation (ratio 1-10).*

DISCUSSION

The results indicate the effect of chemical structure of the emulsifying agent on resin particle charges. At the high latex dilution and low fixed electrolyte

concentration (or more exactly low fixed conductance), the recorded mobilities are considered to be comparable. Although the particles in each latex varied in size the mobility was relatively uniform for all sizes. This was true for each latex whatever the size distribution.

Furthermore as has been shown the variations in percentage solids and pH from sample to sample have little effect. The adsorbed layer of wetting agent or protective colloid would probably be minimal at this dilution. The ionic strength of the solubilized wetting agents or ionizing hydrophilics would resemble that of the uni-univalent electrolyte.

The anionic or cationic nature of the emulsifying agents found in the various dispersions studied governs their electrophoretic mobility. Positive mobilities are of greater magnitude than negative absolute mobilities. This is largely due to quaternary ammonium halides being strongly ionizing whereas long chain organic acids dissociate weakly. This difference in ionic character for soaps and invert soaps is exhibited in media of low pH such as were encountered with all the polyvinyl acetate emulsions included in the studies. Latex 1C exhibits a steeper curve in Fig. 8 than the anionic and nonionic-stabilized emulsions.

Nonionic agents confer low negative mobility. Where mixtures of wetting agents or protective colloids are used in latex formulation, the mobility may be predicted approximately, provided there is no preferential adsorption of one agent to the exclusion of others from the particle interface. If the latter occurs it is probable that the mobility should approach that of an emulsion made using the single, preferentially adsorbed agent.

The existence of any stable polyvinyl acetate latex possessing a particle charge of zero is most doubtful. Commercial emulsions reported as such have been found to be definitely negative. The so-called nonionic materials with anionic character which were studied are assumed to have become negatively charged by hydrogen-bonding or polarization processes or by actual chemical change.

It will be noted that such agents contain, besides the paraffinic chain, a number of hydrophilic points such as ether or hydroxyl groups. Solution in water takes place through the formation of hydrogen bonds or dipole forces between the oxygen atoms and water. Any ions existing in the aqueous media may attach themselves to the polar groups. Those ions which are generally adsorbed are, according to Abramson (3), not too highly hydrated. With sodium chloride, the chloride ion is adsorbed in preference to the sodium ion. This accounts possibly for the occurrence of negative particles when nonionic emulsifiers are used. The most important nonionic protective colloids studied were hydroxyethyl cellulose and polyvinyl alcohol. The latter was polyvinyl acetate hydrolyzed to an extent of about 80%.

Similar explanations for the predominance of negative charge have been postulated by Price and Lewis (10). They concluded that the charge on the particle was composed of two independent parts, both of which were of comparable magnitude. The total charge was assumed due to:

(*a*) adsorption of hydroxyl ions on the surface of the fatty portions of the particle, and

(*b*) ionization of the amphoteric groups.

It is of considerable significance that most emulsion polymerizations of vinyl acetate are promoted by peroxide type catalysts. It is possible that rupture of hydrocarbon chains or oxidation of alcohol and other groups by the strongly oxidizing catalysts is responsible in part for observed negative mobilities. Any carboxyl groups thus interjected into emulsifying agent structure would be capable of ionizing as true anionic components.

The absolute mobility of emulsion 11N (gelatin stabilized) was negative at pH of 4.8. Reference to Abramson's data on the isoelectric point of gelatin (3) indicated that the protective colloid probably used in this latex was deaminized grade. Ordinary gelatin would result in positively charged particles if the emulsion was made at pH less than 4.5, and deaminized gelatin confers positive mobility at pH values not exceeding 4.0.

The only strongly positive emulsions are those based on formulations with quaternary ammonium halides or other nitrogenous surfactants such as amines and amides. All of these materials are synthetic.

Gum arabic and gum tragacanth, examples of naturally occurring emulsifiers, are anionic. Tragacanth is a mixture of several gum acids. The chief constituent of gum arabic is arabic acid to which is attached a member of residues of various hexoses, pentoses, and bioses by means of ethereal oxygen linkages. All such acids occur chiefly as calcium, magnesium, and potassium salts.

Natural materials finding predominant use as stabilizing agents but which were not investigated include alginates, caseinates, pectinates, and lecithin. With little reservation it can be said that these are all either anionic or nonionic with anionic character, and will confer corresponding negative mobility.

Although this investigation has been restricted to polyvinyl acetate latices, it is reasonable to believe that the general picture as set forth should apply to any synthetic resin dispersion. Particle sizes and methods of formulation for all such latices are quite comparable.

ACKNOWLEDGMENT

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NOTES

LIQUID PHASE DEHYDROGENATION OF LAURYL ALCOHOL

By Alexandre Halasz

It is well known that lauryl and other paraffinic alcohols can be easily dehydrogenated to the corresponding aldehydes with various yields by heating them with nickel (1, 2). The yields are lowered by the decomposition of the formed aldehyde and if the reaction is not stopped in time a complete deformylation of the aldehydes to a hydrocarbon occurs.

Reewe and Adkins (4) obtained higher yields by using copper chromite with ethylene under pressure. They obtained a yield of 39% aldehyde and recovered 38% of unreacted alcohol. An inconvenience of this method is the use of ethylene under high pressure; also it does not solve the problem of aldehyde decomposition.

I have noticed that much less decomposition of the aldehyde occurs when the reaction is stopped in time.

For the experiment, pure dodecanol without aldehyde or other impurities was used with 5% copper chromite as catalyst (prepared according to Org. Synth. (3)). The reaction temperature was 250°C. The resulting aldehyde was titrated by the hydroxylamine method and the remaining alcohol determined by acetylation in pyridine. The results can be considered exact within 1% ±. A graph is given below showing the % aldehyde, alcohol, and the sum of the two in the reaction mixture as a function of time.

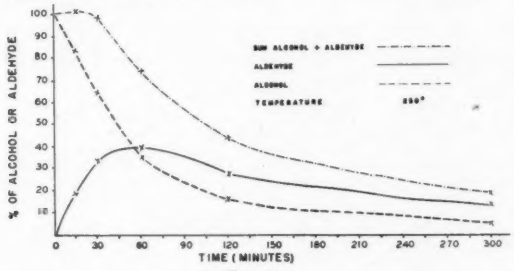

Fig. 1.

As can be seen the maximum yield is attained after 30 min. Up to this time no noticeable decomposition of the aldehyde occurs. The yield of aldehyde at this moment is 34%, but taking in account the unreacted alcohol (65%) the yield is nearly theoretical.

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THE REACTION OF 5-BROMOVANILLIN AND SODIUM METHOXIDE

By R. A. McIvor and J. M. Pepper

Many methods of synthesis of syringaldehyde have been described, the more important of which have been given recently by Pearl (3), while a laboratory procedure has appeared in *Organic Synthesis* (1).

This report concerns preliminary studies of its preparation from vanillin by a two-stage introduction of a methoxyl group. It has recently been reported that universally unsuccessful results were obtained in similar experiments by other workers (4), although they do record a different method for the conversion of vanillin to syringaldehyde. Such a synthesis is of interest because of the ready availability of vanillin as an oxidation product of gymnosperm lignin residues. Of even greater value would be the possibility of thereby converting a mixture of vanillin and syringaldehyde, obtainable from a similar oxidation of hardwood lignin, into the latter chemical.

The reaction studied was the bromination of vanillin to yield 5-bromovanillin which was subsequently treated with methanolic sodium methoxide in the presence of a copper catalyst in an attempt to replace the halogen by the methoxyl grouping. Initial experiments in which the 5-bromovanillin, sodium methoxide, and anhydrous methanol were refluxed, either in the presence or absence of a copper catalyst, resulted only in the recovery of unchanged starting materials. The attempted replacement of the bromine by the fusion of 5-bromovanillin or its triacetate with anhydrous sodium methylate resulted in a very rapid reaction accompanied by a flash and dense white smoke, but a considerable amount of 5-bromovanillin only was recovered from the residue. The nature of this reaction was not further investigated, although qualitative tests showed that ionic bromide and considerable free carbon were formed.

TABLE I

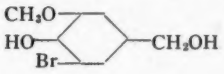
Reaction of 5-bromovanillin and sodium methoxide at high temperatures[a]

Run number	Copper turnings, gm.	Average temp., °C.	Maximum temp., °C.	Time, hr.	Remarks
2	3	163	183	1.8	*b*, *c*
5	7	179	205	2.0	*c*, *d* (3.3 gm.)
6	7	173	186	3.0	*c*, *d* (2.9 gm.)
7	7	150	175	2.0	*c*, *b* (0.7 gm.)
8	7	169	189	1.0	*e*
9	5	158	178	1.5	*e*
10	5	162	172	4	*c*, *f* (5.0 gm.)
11	5	176	180	11.5	*f* (2.5 gm.)
12	5	186	197	9.3	*f* (1.0 gm.)

[a] *For each run, 5-bromovanillin (15 gm.), methanol (250 ml.), and sodium methoxide (10.0 gm.) were used.*
[b] *Crude 5-bromovanillyl alcohol.*
[c] *Filtrate gave positive test for bromide ion.*
[d] *Crude 5-bromovanillyl methyl ether.*
[e] *5-Bromovanillin all recovered.*
[f] *Crude syringaldehyde.*

In order to attempt the replacement at much higher temperatures, a series of runs was made in which the 5-bromovanillin, mixed with anhydrous methanol, sodium methoxide, and copper turnings, was heated in a rocking autoclave. The conditions and results of these experiments are given in Table I. It is believed that the somewhat erratic results obtained under essentially similar conditions were due to the irreproducibility of the copper catalyst. In a forthcoming publication further evidence to support this belief will be presented.

The syringaldehyde was identified by the recrystallization of the crude product from water to yield needles with a melting point of 113° C., undepressed by admixture with an authentic sample (2). The 2,4-dinitrophenylhydrazone melted at 234–235° C., reported m.p. 235.5–236.5 (5). Both the previously unreported compounds, 5-bromovanillyl alcohol (I) (m.p. 129–130° C.) and 5-bromovanillyl methyl ether (II) (m.p. 66° C.), were characterized by an examination of their infrared spectra and the comparison of their



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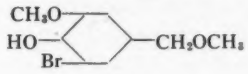

II

ultraviolet absorption spectra and their carbon, hydrogen, and methoxyl analyses with those of the synthetic products. Mixed melting point determinations with these authentic samples showed no depressions. No attempt is being made at this time to explain the mechanism of the formation of these derivatives. Fig. 1 includes, for purposes of comparison, the ultraviolet

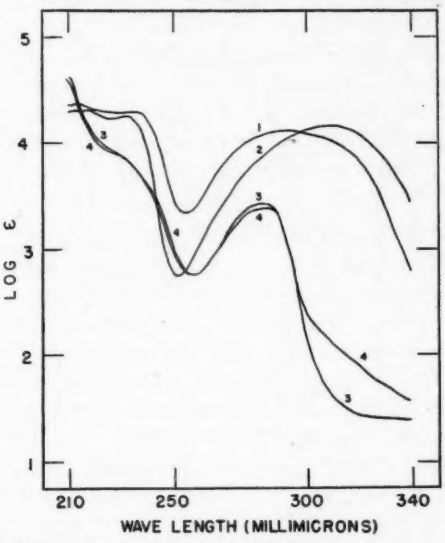

FIG. 1. Ultraviolet absorption spectra (95% ethanol).
1. 5-Bromovanillin.
2. Syringaldehyde.
3. 5-Bromovanillyl alcohol.
4. 5-Bromovanillyl methyl ether.

absorption spectra of 5-bromovanillin, syringaldehyde, 5-bromovanillyl alcohol, and 5-bromovanillyl methyl ether.

Although the preparation of syringaldehyde from 5-bromovanillin appeared quite promising, similar experiments using 5-iodovanillin gave excellent yields. The results of this later work will be presented in a separate article.

EXPERIMENTAL

All ultraviolet absorption spectra were observed on a Beckmann Model DU spectrophotometer in purified 95% ethanol solution. The infrared spectra were determined using a Perkin-Elmer single beam Model 12B spectrophotometer.

5-Bromovanillin

Vanillin (150 gm.) and sodium acetate (50 gm.) were dissolved in glacial acetic acid (350 ml.) and a solution of bromine (180 gm.) in acetic acid (300 ml.) was added slowly with shaking. On completion of the addition, sodium hydrosulphite (15 gm.) dissolved in water (75 ml.) was added, and after most of the excess bromine was reduced the mixture was poured into water (2 liters) containing further sodium hydrosulphite (15 gm.). The 5-bromovanillin readily precipitated; yield 221.8 gm. (97.5%). After recrystallization from ethanol the product melted at 164° C., reported by several workers, 164° C.

5-Bromovanillin-2,4-dinitrophenylhydrazone

The previously unreported 2,4-dinitrophenylhydrazone of 5-bromovanillin was prepared in the usual way; melting point, 295.5–296° C., with decomposition. Calc. for $C_{14}H_{11}N_4O_6Br$: C, 40.89; H, 2.70. Found: C, 40.68; H, 3.64.

5-Bromovanillin Triacetate

5-Bromovanillin (25 gm.) was suspended in acetic anhydride (80 ml.) to which a few drops of concentrated sulphuric acid were added. Solution took place immediately and, on cooling, a precipitate separated. This product was recrystallized from dilute ethanol and dilute acetic acid to give white plates melting at 109° C. Calc. for $C_{14}H_{15}O_7Br$: OCH_3, 8.3; Ac, 34.4. Found: OCH_3, 8.2; Ac, 34.4.

Interaction of 5-Bromovanillin and Sodium Methoxide

The experiments outlined in Table I were carried out in a stainless steel liner, capacity 1080 ml., of an Aminco high pressure hydrogenator, Model No. 406–01 DA. This rocking apparatus served as a high temperature autoclave. The reaction mixture, after cooling and separation of the copper catalyst, was diluted to twice its volume with water and acidified with sulphuric acid. Tarry products often separated at this point, but both the tar and the acidified mixture were exhaustively extracted with ether. This extract, after drying, was concentrated to give an alkali-soluble residue from which the reaction products reported in Table I were isolated. The major part of the syringaldehyde was obtained by re-extraction of the aqueous layers with ethyl acetate.

Identification of 5-Bromovanillyl Alcohol

The crude reaction product was purified to one melting at 129–130° C. by

two recrystallizations from methanol–water and finally by vacuum distillation. The infrared spectrum was determined in the carbonyl and hydroxyl regions in chloroform solution with a calcium fluoride prism. It showed two sharp bands in the OH stretching region at 3601 cm.$^{-1}$ and 3525 cm.$^{-1}$ with molar extinction coefficients of 63 and 138, respectively, the latter believed due to a phenolic hydroxyl group. The ultraviolet spectrum was identical with that of synthetic 5-bromovanillyl alcohol which is given in Fig. 1. A mixed melting point with an authentic specimen, m.p. 132° C., prepared as given below, was undepressed, m.p. 131° C. Calc. for $C_8H_9O_3Br$: mol. wt. 233.1; OCH_3, 13.30; C, 41.22; H, 3.89. Found: mol. wt. (Rast) 235; OCH_3, 12.94; C, 40.71; H, 3.70.

Identification of 5-Bromovanillyl Methyl Ether

The crude reaction product was purified to one melting at 66° C. by two vacuum sublimations. The infrared spectrum was determined in the 3800 cm.$^{-1}$ to 650 cm.$^{-1}$ region in carbon disulphide solution with a sodium chloride prism. It showed only one peak in the hydroxyl region at 3500 cm.$^{-1}$ with a molar extinction coefficient of 94, doubtless due to the phenolic hydroxyl group. The ultraviolet spectrum was identical with that of synthetic 5-bromovanillyl methyl ether which is given in Fig. 1. A mixed melting point with an authentic specimen, m.p. 64–67° C., prepared as given below was undepressed. Calc. for $C_9H_{11}O_3Br$: C, 43.74; H, 4.49. Found: C, 43.83; H, 4.57.

Syntheses of Reference Compounds

5-Bromovanillyl Alcohol

5-Bromovanillin (1.884 gm.) was added to an excess of alkali in water (40 ml.). The sodium salt, which formed immediately, did not dissolve. A solution of sodium borohydride (170 mgm.) in water was added. The faint yellow color of the solution gradually disappeared and the precipitated salt dissolved in 10 min. The solution, after standing at room temperature over night, was acidified with concentrated hydrochloric acid. 5-Bromovanillyl alcohol separated as micro needles. Yield, 1.67 gm. (88%), m.p. 132° C. More of the product could be obtained by extraction of the mother liquors.

5-Bromovanillyl Methyl Ether

5-Bromovanillyl alcohol (133 mgm.) was dissolved in methanol (5 ml.) and concentrated sulphuric acid (1 ml.) added. After the solution had been allowed to stand at room temperature for 36 hr. it was refluxed for five minutes, made alkaline with sodium hydroxide, and extracted with chloroform. The residual aqueous solution was acidified with hydrochloric acid and again extracted with chloroform. This extract was dried and the excess solvent removed to yield a gum (203 mgm.) which readily crystallized in long needles. Vacuum sublimation gave white crystals (110 mgm., 88%), m.p. 64–67° C. Calc. for $C_9H_{11}O_3Br$: OCH_3, 25.12. Found: OCH_3, 24.7.

Absorption Spectra Data

The following ultraviolet and infrared absorption data were recorded.

TABLE II

ULTRAVIOLET ABSORPTION DATA FOR 5-BROMOVANILLIN AND DERIVED COMPOUNDS

Compound	Absorption maxima[a], mμ	Absorption minima[a], mμ
5-Bromovanillin	213.5 (4.36) 234 (4.29) 291 (4.10)	226 (4.28) 255 (3.34)
Syringaldehyde	215 (4.31) 231 (4.25) 308.5 (4.16)	225 (4.23) 251 (2.75)
5-Bromovanillyl alcohol	221 (3.95) 286 (3.42)	257 (2.76)
5-Bromovanillyl methyl ether	221 (3.93) 286 (3.39)	257 (2.75)

[a]*Log molar extinction coefficients given in brackets.*

TABLE III

INFRARED ABSORPTION DATA FOR 5-BROMOVANILLYL METHYL ETHER[a]
ABSORPTION BANDS[b], CM.$^{-1}$

3500 (94)	1237 (139)	914 (34)
2911 (81)	1186 (248)	838 (105)
2824 (70)	1140 (161)	827 (158)
1345 (115)	1100 (185)	788 (41)
1275 (378)	1052 (360)	726 (30)
1250 (112)	961 (39)	

[a]*Determination in carbon disulphide with a sodium chloride prism.*
[b]*Molar extinction coefficients given in brackets.*

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ABSTRACT: **An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

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